



Safety, immunogenicity and efficacy of PfSPZ Vaccine against malaria in infants in western Kenya: a double-blind, randomized, placebo-controlled phase 2 trial

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The radiation-attenuated *Plasmodium falciparum* sporozoite (PfSPZ) vaccine provides protection against *P. falciparum* infection in malaria-naïve adults. Preclinical studies show that T cell-mediated immunity is required for protection and is readily induced in humans after vaccination. However, previous malaria exposure can limit immune responses and vaccine efficacy (VE) in adults. We hypothesized that infants with less previous exposure to malaria would have improved immunity and protection. We conducted a multi-arm, randomized, double-blind, placebo-controlled trial in 336 infants aged 5–12 months to determine the safety, tolerability, immunogenicity and efficacy of the PfSPZ Vaccine in infants in a high-transmission malaria setting in western Kenya ([NCT02687373](#)). Groups of 84 infants each received 4.5×10^5 , 9.0×10^5 or 1.8×10^6 PfSPZ Vaccine or saline three times at 8-week intervals. The vaccine was well tolerated; 52 (20.6%) children in the vaccine groups and 20 (23.8%) in the placebo group experienced related solicited adverse events (AEs) within 28 d postvaccination and most were mild. There was 1 grade 3-related solicited AE in the vaccine group (0.4%) and 2 in the placebo group (2.4%). Seizures were more common in the highest-dose group (14.3%) compared to 6.0% of controls, with most being attributed to malaria. There was no significant protection against *P. falciparum* infection in any dose group at 6 months (VE in the 9.0×10^5 dose group = −6.5%, $P = 0.598$, the primary statistical end point of the study). VE against clinical malaria 3 months after the last dose in the highest-dose group was 45.8% ($P = 0.027$), an exploratory end point. There was a dose-dependent increase in antibody responses that correlated with VE at 6 months in the lowest- and highest-dose groups. T cell responses were undetectable across all dose groups. Detection of V δ 2⁺V γ 9⁺ T cells, which have been correlated with induction of PfSPZ Vaccine T cell immunity and protection in adults, were infrequent. These data suggest that PfSPZ Vaccine-induced T cell immunity is age-dependent and may be influenced by V δ 2⁺V γ 9⁺ T cell frequency. Since there was no significant VE at 6 months in these infants, these vaccine regimens will likely not be pursued further in this age group.

Malaria is a mosquito-borne parasitic disease responsible for an estimated 229 million cases and 409,000 deaths in 2019, primarily from *P. falciparum* among sub-Saharan African children¹. Between 2000 and 2015 there was a substantial reduction in global malaria cases and deaths due to the implementation of malaria control efforts². However, despite these efforts, since 2015 the number of annual cases and deaths has remained stable, highlighting the urgent need to develop vaccines to prevent malaria.

The most clinically advanced vaccine against *P. falciparum*, RTS,S, is a subunit vaccine consisting of a single recombinant protein, the *P. falciparum* circumsporozoite protein (PfCSP), administered with

the adjuvant AS01. In a phase 3 clinical trial in 5–17-month-old infants, three vaccinations with RTS,S/AS01 conferred 51.3% vaccine efficacy (VE) against all episodes of *P. falciparum* clinical disease at one year³; 4 vaccinations given over 21 months conferred 36.3% VE over 4 years⁴. Protection by RTS,S is believed to be primarily antibody-mediated⁵.

The PfSPZ Vaccine uses a different approach consisting of live (metabolically active), nonreplicating, radiation-attenuated, aseptic, purified, cryopreserved *P. falciparum* sporozoites (SPZ). Initial studies showed an approximate 60–100% VE up to 14 months against homologous (the same parasite strain is used for challenge

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as that used for the vaccine) controlled human malaria infection (CHMI)⁶ in malaria-naïve US adults^{6,7}. A notable feature for the effectiveness of the PfSPZ Vaccine is the requirement for administration by direct venous inoculation (DVI)^{6,8}. This route induces circulating PfCSP-specific antibodies as well as circulating and liver-resident T cell responses^{6,7,9,10}. Although antibodies specific to PfCSP have been shown to mediate short-term VE^{11–13}, numerous preclinical animal models of malaria infection have demonstrated that PfSPZ-specific T cells are required for durable sterilizing protection^{8,14,15}. In malaria-naïve humans, multifunctional CD4⁺ T cell cytokine-producing cells in the blood have also been associated with protection by PfSPZ Vaccines^{7,10}. Priming of a protective CD4 and CD8 T cell response after PfSPZ Vaccine may be mediated in part by γδ T cells¹⁶. The frequency of Vδ2⁺Vγ9⁺ T cells at the time of initial PfSPZ immunization is correlated with the induction of PfSPZ-specific T cells and a protective outcome in adults⁷. γδ T cells are also required at the time of immunization of mice with rodent malaria sporozoites for the induction of protective immunity¹⁶.

In advancing the PfSPZ Vaccine to malaria-endemic countries, an initial study showed that in Malian adults, VE against malaria infection as detected by microscopic examination of thick blood smears was 52% and 29% by time-to-event (1 – hazard ratio) and proportional analysis (1 – risk ratio), respectively over 6 months of natural, intense malaria exposure during the rainy season¹⁷. Of note, antibody responses were 30.6 times and T cell responses at least 5 times lower in malaria-exposed African adults than in malaria-naïve North American volunteers receiving the same vaccine regimen^{9,17–19}. These data suggest that previous malaria exposure may limit the immune responses to the vaccine, probably through a variety of regulatory mechanisms²⁰. This led to the hypothesis that vaccinating infants, who have less previous malaria exposure in malaria-endemic regions, may show enhanced immune responses and protection. Moreover, African infants and young children continue to be at greatest risk of malaria mortality and thus constitute a critical age group to study.

In this study, we report the results of a phase 2 randomized controlled trial using normal saline as the comparator to assess the safety, immunogenicity and VE after DVI of PfSPZ Vaccine in 5–12-month-old infants in Kenya. The data presented in this study have important implications for age-dependent responses to the PfSPZ Vaccine and provide a hypothesis for how the frequency of Vδ2⁺Vγ9⁺ T cells may be critical for influencing T cell priming by the PfSPZ Vaccine.

Results

Study population. A total of 548 infants aged 5–12 months were screened for eligibility over 12 weeks from January 2017 to April 2017; 337 were randomized. Of 211 excluded infants, parents of 106 withdrew consent before screening, 88 failed screening (primary reasons: abnormal hematological parameters ($n=23$), acute/chronic malnutrition ($n=17$), abnormal electrocardiogram (ECG) readings ($n=16$)) and 17 were eligible but not enrolled for various reasons (Extended Data Fig. 1). One randomized infant was withdrawn by the mother after three unsuccessful attempts to access veins for vaccination. Vaccination 1 was given to 336 infants in 4 groups of 84 participants each receiving 4.5×10^5 , 9.0×10^5 and 1.8×10^6 PfSPZ or normal saline placebo. Initial sample size calculations were powered assuming 30% infection in the control arm; however, this was approximately 67% at six months, effectively reducing the sample required to detect a VE of 60%. A total of 317 infants received dose 3; 291 completed the 12-month follow-up period in August 2018 and 260 were included in the according-to-protocol cohort at month 6 (Extended Data Fig. 1). One major protocol deviation occurred, whereby an enrolled and vaccinated child was later found to be human immunodeficiency virus (HIV)-exposed and taking co-trimoxazole prophylaxis, an enrollment contraindication; this

was reported immediately to the institutional review boards and the child was withdrawn from the study. Baseline characteristics were similar for age, sex, ethnicity and weight-for-height among the treatment groups (Supplementary Table 2). At the time of the first and second immunizations, 25 out of 260 (9.6%) and 20/260 (7.7%) infants, respectively were positive for *P. falciparum* by blood smear (BS) and 40 out of 257 (15.6%) and 42 out of 259 (16.2%) by PCR (Supplementary Table 3). All infants, regardless of study arm, were administered a full 3-d treatment course with artemisinin combination therapy before the third immunization to allow accurate measurement of new infections.

Safety. Overall, 51.6% of vaccinees experienced a solicited AE (20.6% considered vaccine-related) after at least 1 vaccination versus 52.4% of placebo recipients (23.8% considered injection-related) (Table 1). Local solicited AEs were considered to be intervention-related and occurred in 11.9% of vaccine recipients and 16.7% of placebo recipients, with 1 of grade 3 severity in the 4.5×10^5 dose group and 1 in the placebo group. Solicited systemic AEs were common and affected 45.6% of vaccinees and 45.2% of placebo recipients (Table 1). The most common solicited systemic AE was fever. Related fever with no alternate etiology occurred in 7.5% of vaccinees and 4.8% of placebo recipients (1 of grade 3 severity in the placebo group) and was more common in participants in the highest-dose group (Supplementary Table 4). The majority of solicited AEs were mild to moderate (Supplementary Table 5). Unsolicited AEs within 28 d of vaccination occurred in 98.8% of vaccine and 95.2% of placebo recipients, with 6.0% and 4.8%, respectively considered related to the intervention (Table 1).

Laboratory indicators of safety on day 8 postvaccinations 1 and 2 and on day 14 postvaccination 3 showed similar levels of abnormalities between the vaccine and placebo groups (Supplementary Table 6). Anemia was common in all groups; about half of the participants who had reduced hemoglobin levels postvaccination had preexisting anemia at screening.

SAEs occurred in 15.1% of vaccine recipients and 9.5% of placebo recipients (Table 1), including 16 out of 84 (19.0%) and 13/84 (15.5%) participants in the 1.8×10^6 and 9×10^5 PfSPZ groups compared to 9 out of 84 (10.7%) and 8 out of 84 (9.5%) in the 4.5×10^5 PfSPZ and placebo groups, respectively (Supplementary Tables 7 and 8). All SAEs were considered unrelated to the study intervention because a definite alternate etiology was identified. There was one death during the study period in a placebo participant due to a bee swarm attack more than 6 months after vaccination 3.

Seizures, which were part of the clinical presentation leading to several hospitalizations, were also more common in vaccine recipients (9.9%, 95% confidence interval (CI)=6.7–14.5) than placebo recipients (6.0%, 95% CI=2.2–14.0), occurring in 12 out of 84 (14.3%) of the highest-dose group compared with 7 out of 84 (8.3%) in the 9.0×10^5 dose group, 6 out of 84 (7.1%) of the 4.5×10^5 dose group and 5 out of 84 (6.0%) in the placebo group (Table 1). Most of the febrile seizures in all dose groups were related to malaria, with nine malaria-related seizures in the highest-dose group, five each in the two lower-dose groups and four in the placebo group (Supplementary Table 7). Two of the three children diagnosed with epilepsy had a total of two and three seizures, which were controlled by anticonvulsants; they were seizure-free and off medication at the time of the post-close-out interview. One child had infantile spasms and severe developmental delay; mild developmental delay was retrospectively determined to have been preexisting before vaccination. MRI was suggestive of changes occurring shortly after birth but etiology was not clear. Out of concern for these findings relating to hospitalizations and seizures, a post-close-out interview was conducted between 14 and 21 months after the study end, focusing on parents/caregivers of 309 children who had received all 3 vaccinations, had not withdrawn consent and were not excluded from

Table 1 | Summary of AEs^a

| | 4.5 × 10 ⁵ PfSPZ (n = 84) | | 9 × 10 ⁵ PfSPZ (n = 84) | | 1.8 × 10 ⁶ PfSPZ (n = 84) | | All vaccine (n = 252) | | All placebo (n = 84) | |
|-------------------------------------------------------------------------------------------------------|-----------------------------------------|-------------|------------------------------------|-------------|--------------------------------------|-------------|-----------------------|--------------|----------------------|-------------|
| | All AEs | Related AEs | All AEs | Related AEs | All AEs | Related AEs | All AEs | Related AEs | All AEs | Related AEs |
| Participants with at least one solicited AE, n (%) | 42 (50.0) | 14 (16.7) | 43 (51.2) | 16 (19.0) | 45 (53.6) | 22 (26.2) | 130 (51.6) | 52 (20.6) | 44 (52.4) | 20 (23.8) |
| Participants with a solicited grade 3 AE, n (%) | 7 (8.3) | 1 (1.2) | 8 (9.5) | 0 (0) | 7 (8.3) | 0 (0) | 22 (8.7) | 1 (0.4) | 5 (6.0) | 2 (2.4) |
| Participants with at least one solicited local AE ^b | 7 (8.3) | 7 (8.3) | 8 (9.5) | 8 (9.5) | 15 (17.9) | 15 (17.9) | 30 (11.9) | 30 (11.9) | 14 (16.7) | 14 (16.7) |
| Participants with a solicited local grade 3 AE, n (%) | 1 (1.2) | 1 (1.2) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1 (0.4) | 1 (0.4) | 1 (1.2) | 1 (1.2) |
| Participants with at least one solicited systemic AE ^c | 40 (47.6) | 7 (8.3) | 41 (48.8) | 9 (10.7) | 34 (40.5) | 8 (9.5) | 115 (45.6) | 24 (9.5) | 38 (45.2) | 7 (8.3) |
| Participants with a solicited systemic grade 3 AE, n (%) | 6 (7.1) | 0 (0) | 8 (9.5) | 0 (0) | 7 (8.3) | 0 (0) | 21 (8.3) | 0 (0) | 4 (4.8) | 1 (1.2) |
| Participants with at least one unsolicited AE within 28 d of immunization, n (%) | 83 (98.8) | 5 (6.0) | 82 (97.6) | 5 (6.0) | 84 (100.0) | 5 (6.0) | 249 (98.8) | 15 (6.0) | 80 (95.2) | 4 (4.8) |
| Total number of unsolicited AEs within 28 d of immunization, n (maximum severity grade) | 445 (6 of grade 3) | 5 (grade 1) | 426 (8 of grade 3) | 5 (grade 1) | 452 (12 of grade 3) | 5 (grade 1) | 1,323 (26 of grade 3) | 15 (grade 1) | 431 (6 of grade 3) | 6 (grade 1) |
| Participants with an unsolicited grade 3 AE within 28 d of immunization, n (%) | 5 (6.0) | 0 (0) | 7 (8.3) | 0 (0) | 10 (11.9) | 0 (0) | 22 (8.7) | 0 (0) | 5 (6.0) | 0 (0) |
| Participants experiencing an SAE during the entire study period, n (%) | 9 (10.7) | 0 (0) | 13 (15.5) | 0 (0) | 16 (19.0) | 0 (0) | 38 (15.1) | 0 (0) | 8 (9.5) | 0 (0) |
| Total number of SAEs | 10 | 0 | 15 | 0 | 19 | 0 | 44 | 0 | 9 | 0 |
| Participants with at least one seizure during the entire study period (AE of special interest), n (%) | 6 (7.1) | 0 (0) | 7 (8.3) | 0 (0) | 12 (14.3) | 0 (0) | 25 (9.9) | 0 (0) | 5 (6.0) | 0 (0) |
| Participants with at least one febrile seizure, n (%) | 6 (7.1) | 0 (0) | 6 (7.1) | 0 (0) | 10 (11.9) | 0 (0) | 22 (8.7) | 0 (0) | 5 (6.0) | 0 (0) |
| Participants with at least one afebrile seizure, n (%) | 0 (0) | 0 (0) | 1 (1.2) | 0 (0) | 2 (2.4) | 0 (0) | 3 (1.2) | 0 (0) | 0 (0) | 0 (0) |

^aThere were no significant differences between any groups for any AEs. ^bLocal solicited AEs included pain, pruritis, swelling, redness (erythema), bruising and induration at injection site. ^cSystemic solicited AEs included allergic rash/urticaria/generalized pruritus, drowsiness, irritability/fussiness, inability to eat and fever.

Table 2 | VE against parasitemia (primary end point) and clinical malaria (exploratory) end points at 3, 6, 9 and 12 months, among the according-to-protocol cohort

| | 3 months | 6 months (primary end point) | | 9 months | | 12 months | | P |
|-----------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------|------------------------------|------------------------------------------------------------|------------------------------------------------------------------|------------------------------------------------------------|------------------------------------------------------------------|------------------------------------------------------------|-----------------------|
| | | Proportion infected, n/N (%) | VE (95% CI) n/N (%) / P value incidence ^a | Proportion infected, n/N (%) / P value incidence ^a | VE (95% CI) n/N (%) / P value incidence ^a | Proportion infected, n/N (%) / P value incidence ^a | VE (95% CI) n/N (%) / P value incidence ^a | |
| Any <i>P. falciparum</i> parasitemia (>0 parasites μl^{-1}), proportional efficacy | | | | | | | | |
| Placebo | 41/71 (57.8) | ref | 45/68 (66.2) | ref | 47/67 (70.2) | ref | 51/67 (76.1) | ref |
| 4.5 \times 10 ⁵ PfSPZ | 34/67 (50.8) | 12.1 (-19.6, 35.5) | 0.412 (65.6) | 42/64 (65.6) | 0.8 (-26.8, 22.4) | 0.947 (81.0) | 51/63 (52) | -15.4 (-40.5, 52) |
| 9.0 \times 10 ⁵ PfSPZ | 32/65 (49.2) | 14.7 (-17.1, 37.9) | 0.324 (70.5) | 43/61 (70.5) | -6.5 (-34.7, 15.8) | 0.598 (78.0) | 46/59 (96) | -11.1 (-36.7, 96) |
| 1.8 \times 10 ⁶ PfSPZ | 28/68 (41.2) | 28.7 (-0.1, 49.6) | 0.056 (58.2) | 39/67 (58.2) | 12.0 (-14.6, 32.5) | 0.342 (65.2) | 43/66 (65.2) | 7.1 (-17.6, 26.6) |
| Any <i>P. falciparum</i> parasitemia (>0 parasites μl^{-1}), time-to-event analysis | ref | ref | ref | ref | ref | ref | ref | ref |
| Placebo | 4.5 \times 10 ⁵ PfSPZ | 20.7 (-25.0, 49.7) | 0.318 (43.2) | 13.5 (-31.8, 43.2) | 0.500 (29.7) | -4.51 (-55.4, 29.7) | 0.83 (-19.4, 18.4) | 0.36 (-74.7, 18.4) |
| 9.0 \times 10 ⁵ PfSPZ | 21.4 (-24.8, 50.5) | 0.307 (36.4) | 3.3 (-46.8, 36.4) | 0.874 (29.0) | -6.65 (-60.2, 29.0) | 0.76 (-21.4, 17.6) | -21.4 (-79.0, 17.6) | 0.33 (-79.0, 17.6) |
| 1.8 \times 10 ⁶ PfSPZ | 41.1 (47.63.6) | 0.031 (54.5) | 30.1 (-7.4, 54.5) | 0.103 (51.3) | 26.4 (-11.3, 51.3) | 0.15 (9.6, 38.3) | -32.5 (-32.5, 38.3) | 0.61 (-32.5, 38.3) |
| Clinical malaria (temperature \geq 37.5°C and <i>P. falciparum</i> parasitemia \geq 5,000 parasites μl^{-1}), all episodes | | | | | | | | |
| Placebo | 34/179.7 (0.19) | ref | 66/375.6 (0.18) | ref | 83/570.5 (0.15) | ref | 106/780.1 (0.14) | ref |
| 4.5 \times 10 ⁵ PfSPZ | 22/168.2 (0.13) | 35.9 (-9.8, 62.5) | 0.106 (0.13) | 47/353.5 (44.7) | 19.8 (-16.1, 44.7) | 0.24 (0.13) | 72/536.5 (29.8) | 3.5 (-32.7, 29.8) |
| 9.0 \times 10 ⁵ PfSPZ | 30/164.0 (0.18) | 4.2 (-56.6, 41.4) | 0.865 (0.15) | 50/336.9 (38.2) | 11.5 (-26.8, 38.2) | 0.5 (0.14) | 70/502.4 (26.2) | 0.4 (-34.5, 26.2) |
| 1.8 \times 10 ⁶ PfSPZ | 19/171.5 (0.11) | 45.8 (6.9, 68.5) | 0.027 (0.12) | 43/370.1 (52.2) | 28.6 (-6.8, 52.2) | 0.1 (0.12) | 66/562.0 (38.9) | 13.5 (-22.6, 38.9) |
| Clinical malaria (temperature \geq 37.5°C and <i>P. falciparum</i> parasitemia \geq 5,000 parasites μl^{-1}), time to first episode | ref | ref | ref | ref | ref | ref | ref | ref |
| Placebo | 4.5 \times 10 ⁵ PfSPZ | 38.4 (-11.0, 65.8) | 0.107 (54.7) | 26.4 (-19.8, 54.7) | 0.220 (44.4) | 13.0 (-36.1, 44.4) | 0.54 (-4.3, 31.8) | 0.85 (-59.7, 31.8) |
| 9.0 \times 10 ⁵ PfSPZ | 71 (-59.6, 45.9) | 0.791 (0.71) | 8.5 (-47.5, 43.2) | 0.71 (0.74) | -7.7 (-68.0, 30.9) | -0.74 (-19.8, 51.7) | -28.5 (-95.8, 15.6) | 0.24 (-49.6, 35.3) |
| 1.8 \times 10 ⁶ PfSPZ | 40.2 (-7.7, 66.8) | 0.087 (59.4) | 33.7 (-8.4, 59.4) | 0.10 (23.9) | 23.9 (-19.8, 51.7) | 0.24 (1.6) | 0.94 (-49.6, 35.3) | 0.94 (-49.6, 35.3) |

^aProportion for parasitemia outcomes; incidence in terms of clinical episodes per person-months for clinical malaria outcomes.

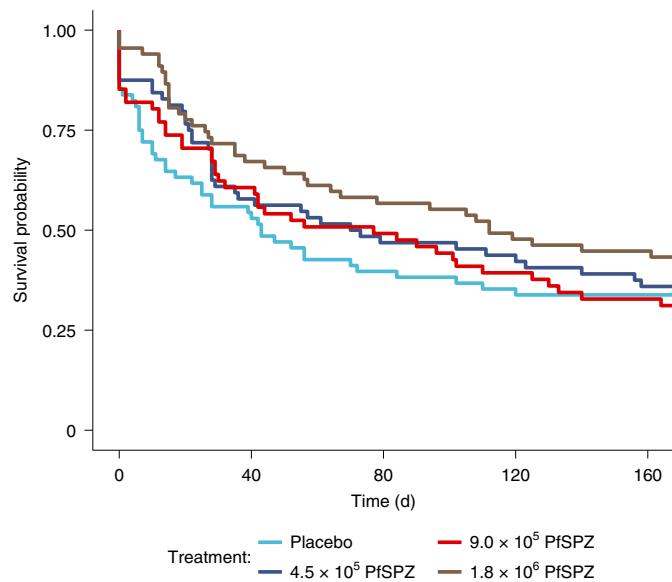


Fig. 1 | Time to first/only parasitemia. Kaplan-Meier survival analysis of time to first episode or only parasitemia over six months by treatment group in the 6-month according-to-protocol cohort.

analysis (one HIV-exposed participant was excluded after vaccination 3). Caregivers of 272 out of 309 children (88%) were reached and confirmed that their child was alive and well; of these, 255 parents were interviewed during the dissemination meetings. There was no difference in the number of hospital admissions, severe malaria or seizures between treatment groups in the interval between study close-out and the survey (Supplementary Table 7).

VE. None of the dose groups showed statistically significant ($P < 0.05$) VE against the presence of parasitemia at the primary 6-month end point by either proportional or time-to-event analyses (Table 2), although the highest-dose group showed the highest VE up to this time point (Fig. 1) at 12.0% (95% CI = −14.6%, 32.5%) by proportional analysis and 30.1% (95% CI = −7.4%, 54.5%) by time-to-event analysis (Table 2). VE against the presence of parasitemia was higher in all groups at 3 months, reaching statistical significance ($P = 0.03$) for the according-to-protocol population in the highest-dose group by time-to-event analysis (VE 41.1%; 95% CI = 4.7%, 63.6%) and borderline significance ($P = 0.06$) by proportional analysis (VE 28.7%; 95% CI = −0.10%, 49.6%). Thereafter, VE declined across the follow-up period in all dose groups (Table 2 and Extended Data Fig. 2). Similarly, significant VE against clinical malaria (primary definition: fever $\geq 37.5^\circ\text{C}$ and *P. falciparum* parasite density $\geq 5,000$ parasites per microliter) was only observed in the highest-dose group and was 45.8% (95% CI = 6.9%, 68.5%) for all episodes of clinical malaria at 3 months ($P = 0.027$) in the according-to-protocol population. However, there was no significant VE at 6 months against clinical malaria in any of the dose groups, using time to first episode and all episodes for either the primary definition of clinical malaria or the secondary case definitions of clinical malaria (Supplementary Table 9 and Extended Data Fig. 3).

Immunogenicity. *PfSPZ* Vaccine does not induce *PfSPZ* T cell responses in infants. In this study, we used a new 28-color flow cytometry panel to assess vaccine-specific T cell responses 2 weeks after the 3rd vaccination in peripheral blood mononuclear cells (PBMCs) after in vitro restimulation. This panel included multiple markers to delineate multiple helper T cell subsets (T_{H1} , T_{H2} , T_{FH}),

$\gamma\delta$ T cell subsets, T cell activation and differentiation and T cell function (Supplementary Tables 10 and 11 and Extended Data Fig. 4). There were low-to-undetectable *PfSPZ*-specific CD4 and CD8 T cell interferon- γ (IFN- γ), interleukin-2 (IL-2) or tumor necrosis factor- α (TNF- α) responses, which have been associated with protection in adults^{7,10}; these were not different from pre-vaccine responses (Fig. 2a,b). Furthermore, there were no CD4 T cell responses including other cytokines such as IL-8, IL-17, IL-21 and T_{H2} cytokines (Extended Data Fig. 5a–d). The lack of *PfSPZ* vaccine-specific T cell responses in infants was not due to an intrinsic inability to produce cytokines since stimulation with phorbol myristate acetate (PMA)/ionomycin induced nonspecific robust responses (Fig. 2a,b). Furthermore, in the batch analysis of PBMCs from vaccinated infants, PBMCs from a *PfSPZ*-vaccinated adult sample were included as an internal control to generate T cell responses by stimulation with *PfSPZ* in vitro and these cells showed an antigen-specific cytokine response (Extended Data Fig. 5e).

Infants have a low frequency of V82 T cells. To better understand the lack of vaccine-induced CD4 and CD8 $\alpha\beta$ T cell responses in infants, we extended the analysis to other populations of T cells. Previous studies by us and others demonstrated that $\gamma\delta$ T cells expand after *PfSPZ* vaccination⁷ or natural malaria infection^{21–23} and that the frequency of V82 $\gamma\delta$ T cells at the time of vaccination correlates with protection^{7,16}. However, in the present study, only a minor proportion (approximately 22%) of $\gamma\delta$ T cells were V82 (Fig. 3a), which is consistent with previously reported baseline for this age group²⁴. Furthermore, after *PfSPZ* vaccination there was no change in the frequencies or activation of each $\gamma\delta$ T cell subset (Fig. 3a,b). We next assessed the functional cytokine response of $\gamma\delta$ T cells after *PfSPZ* vaccination. Like the finding with $\alpha\beta$ T cells, there was no notable vaccine-induced functional cytokine response by $\gamma\delta$ T cells after the third vaccination in infants (Fig. 3c,d), in contrast to the positive control using PBMCs from an adult who received the *PfSPZ* Vaccine (Fig. 3c and Extended Data Fig. 5e). Of note, among infants who generated a functional cytokine response above 0.1% of all $\gamma\delta$ T cells, after the final vaccination time point, most of these cells were V82⁺ (Fig. 3d). The finding that V82 T cells represent only a minor subset of the $\gamma\delta$ T cell repertoire in infants is notable since the frequency and function of such cells have been shown to correlate with protection against malaria in children living in endemic regions²³. Thus, based on the large number of infants in the placebo and vaccine groups that were prospectively followed for infection over a year, we determined how the frequencies of $\gamma\delta$ T cell subsets during the course of the study influenced the risk of infection in all individuals. The frequencies of V81 and V82 T cells were determined at the pre-vaccination time point and correlated with infection at 3 different time points in the study: during the vaccination phase (dVax); at 3 months; and at 6 months for the respective according-to-protocol cohorts (Extended Data Fig. 6). Infants with the highest frequencies of V82 T cells at the pre-vaccination time point had significantly lower frequency of malaria infection at all three time points. By contrast, infants with the highest V81 frequencies at the pre-vaccination time point had a higher frequency of malaria infection across all time points. These data demonstrate that V82 T cell frequencies in these infants correlate with protection against malaria infection independent of vaccination.

Overall, based on these data, we hypothesized that the limited magnitude and functional capacity of V82 T cells in infants may be a mechanism for the lack of T cell responses after immunization with the *PfSPZ* Vaccine in infants.

Age-dependent association of V82 T cells and T cell immunity by *PfSPZ* Vaccine. To determine if there was an association between the frequency of V82 T cells and the magnitude of *PfSPZ*-specific CD4 T cell responses, we assessed the frequency of $\gamma\delta$ T cell

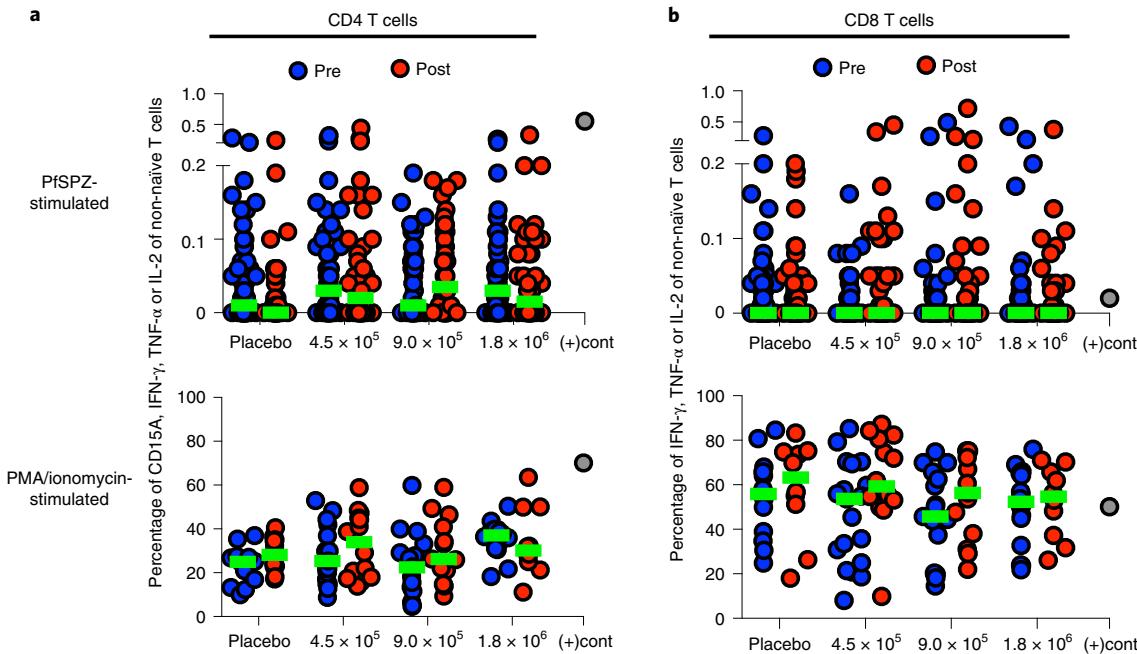


Fig. 2 | PfSPZ Vaccine-induced adaptive immune response. **a,b**, T cell response to the PfSPZ Vaccine. Percentage of non-naïve CD4 (**a**) or CD8 (**b**) T cells expressing CD154, IFN- γ , IL-2 or TNF- α at pre-vaccination (blue) or two weeks after the third vaccination (red) of the indicated dose of the PfSPZ Vaccine. Results are the percentage of cytokine-producing cells after incubation with PfSPZ (top) or PMA/ionomycin (bottom) minus the percentage of cytokine-producing cells after incubation with vaccine diluent (medium with 1% human serum albumin). A positive control sample from a single vaccinated adult was included in each batch analysis and the median value across all batches is indicated by the gray symbol. The green bars indicate the median values within each group. Differences at each time point between pre- and postvaccination groups were assessed using multiple t-tests with Holm-Sidak's correction for multiple comparisons.

subsets in PBMCs from a separate immunogenicity study in Tanzania¹⁹ (NCT02613520) from participants of different age groups that received the PfSPZ Vaccine. The distribution of $\gamma\delta$ T cell subsets in these individuals revealed an age-dependent shift. The frequency of V82 T cells represented only a small portion of the infant $\gamma\delta$ T cell repertoire (Fig. 3e), similar to what we observed in the current study (Fig. 3a). Interestingly, there was a significant correlation between the frequency of V82 T cells and PfSPZ-specific CD4 T cells across all age groups (Fig. 3f).

PfSPZ Vaccine induces potent PfCSP antibody responses that correlate with short-term protection. To assess the humoral immune response, we first measured antibodies to PfCSP, the major protein expressed on PfSPZ that can have a protective role against malaria infection^{7,25}. Two weeks after the 3rd vaccination, 94.0% and 89.5% of vaccinees and 12.7% and 12.7% of controls had increased IgG and IgM antibodies, respectively to PfCSP compared to preimmunization levels and which met criteria for seroconversion (Supplementary Table 12). There was a dose-dependent increase in IgG and IgM antibody responses (Extended Data Fig. 7) and a significantly greater rate of seroconversion and net increase in IgG and IgM antibodies in infants who remained uninfected (protected) than in those who became infected at the 3- and 6-month time points (Fig. 4a, Extended Data Fig. 8 and Supplementary Tables 12 and 13). This difference was significant for two of the three dosage groups for IgG and all three dosage groups for IgM antibodies for the 6-month follow-up (Fig. 4b) and two of three dosage groups for the 3-month follow-up (Extended Data Fig. 8b). To extend this analysis, a labeled PfCSP-specific probe was used to measure antigen-specific memory B cells by flow cytometry (Extended Data Fig. 9). There was a dose-dependent increase in the PfCSP-specific memory B cell response from the pre-vaccination time point to two weeks after the

third vaccination. Short-term protection in the 1.8×10^6 dose group was associated with an increase in the PfCSP-specific memory B cell response (Fig. 4c).

Discussion

This study, the first phase 2 clinical efficacy trial of the PfSPZ Vaccine in infants, showed that administration of the PfSPZ Vaccine by DVI was well tolerated in infants. Infants received a total of 979 DVI inoculations and there were no significant differences in solicited or unsolicited AEs between vaccinees and saline controls. However, there was a higher number of hospitalizations and seizures in the two highest-dose groups; these differences were not statistically significant but the study was not powered enough to detect such differences. The imbalance in febrile seizures was primarily driven by malaria-related seizures. Because of this finding, interviews with the caregivers of 82.5% of participants who had received all 3 vaccine doses were conducted 14–21 months after the end of the study; at this time, there was no further imbalance in admissions or occurrence of seizures between the vaccine groups and the placebo group. Overall, these data suggest that doses up to 1.8×10^6 of PfSPZ Vaccine can be considered safe in this age group, extending the large dataset of safety and tolerability of this vaccine in adults and children^{17–19,26} down to 5-month-old infants. Ongoing and future trials of the PfSPZ Vaccine in older children are including seizures as an AE of special interest to further evaluate the observed imbalance in SAEs and seizures using a larger sample size.

While there was some VE against *P. falciparum* infection and clinical malaria in the highest-dose group during the first three months after the last dose of vaccine, there was no significant protection in any dose group at six months, the primary statistical end point of the study. The VE of 45.8% against all clinical malaria during the 3 months after last dose and of 28.6% during the 6 months

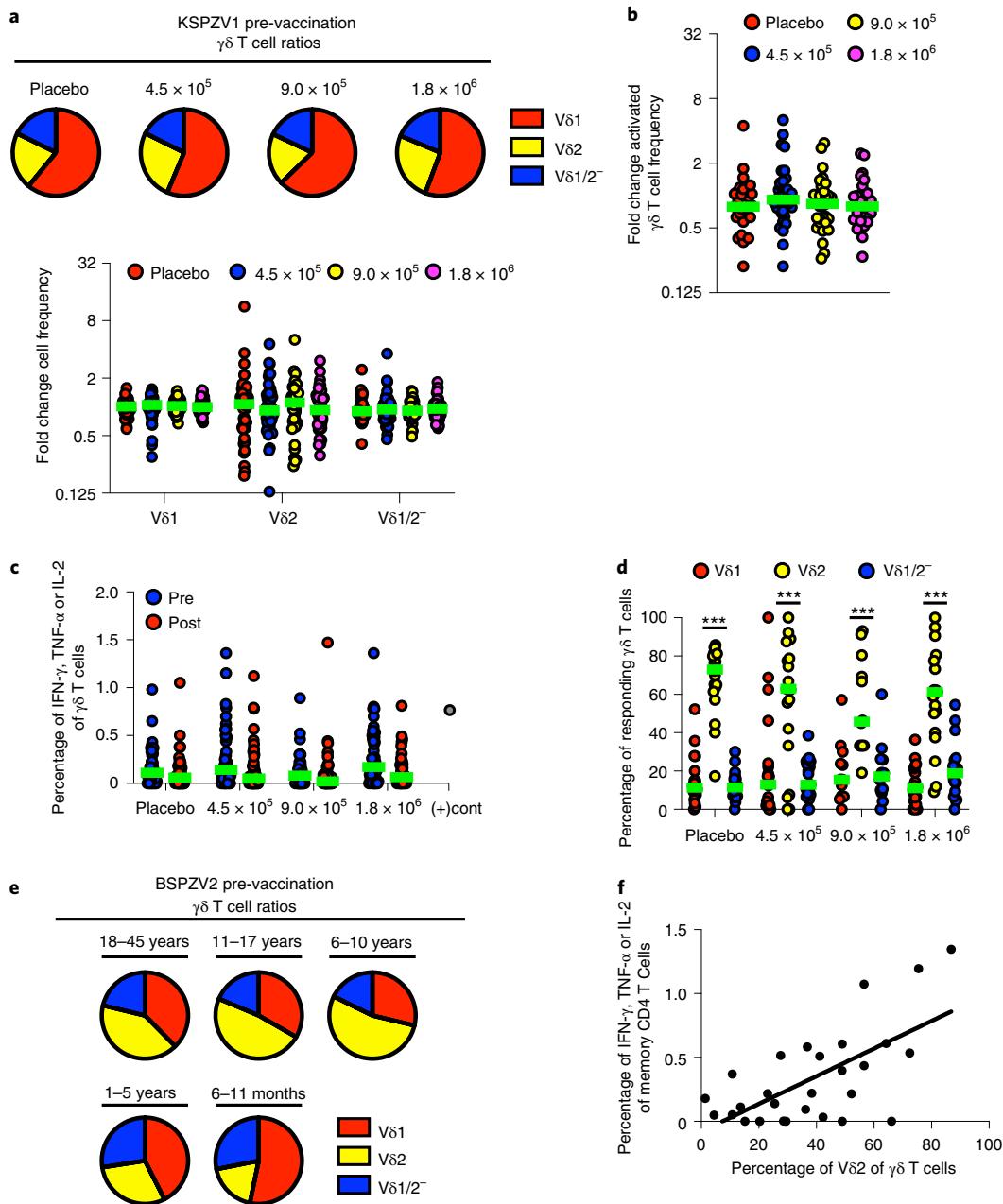


Fig. 3 | PfSPZ Vaccine-induced γδ T cell response. **a–d**, γδ T cell analysis. **a**, Pre-vaccination ratios of γδ T cell subsets from the indicated vaccine groups (top) and fold change of γδ T cell subset frequencies from pre-vaccination to two weeks after the third vaccination within the indicated vaccine dose groups (bottom). **b**, Fold change of activated (CD38 $^{+}$ HLA-DR $^{+}$) γδ T cell frequencies from pre-vaccination to two weeks after the third vaccination within the indicated vaccine dose groups. The green bars indicate the median values within each group; differences among and within vaccine groups were assessed by two-way analysis of variance (ANOVA) using Tukey's test to correct for multiple comparisons. **c**, γδ T cell response to the PfSPZ Vaccine. Percentage of all γδ T cells expressing IFN- γ , IL-2 or TNF- α at preimmunization (blue) or two weeks after the third immunization (red) of the indicated dose of PfSPZ Vaccine. Results are the percentage of cytokine-producing cells after incubation with PfSPZ minus the percentage of cytokine-producing cells after incubation with vaccine diluent. The gray symbol indicates the median value for positive control sample within each batch as in Fig. 1. Differences at each time point between pre- and postvaccination groups were assessed using multiple *t*-tests with Holm–Sidak's correction for multiple comparisons. **d**, γδ T cells from the postvaccination time point within each vaccine group that had a functional response >0.1% from **c** were subdivided to show the frequencies of individual γδ subsets responsible for cytokine production. Differences among and within vaccine groups were assessed by two-way ANOVA using Tukey's test to correct for multiple comparisons. *** P <0.0001. **e,f**, γδ T cell data from an age de-escalation PfSPZ Vaccine trial in Bagamoyo, Tanzania (BSPZV2). **e**, Pre-vaccination ratios of γδ T cell subsets from individuals within the indicated age groups. **f**, Correlation between frequencies of functional CD4 T cells and Vδ2 T cells after the first vaccination in individuals from the BSPZV2 trial. Linear regression was calculated; r^2 =0.427 and the slope has a non-zero P <0.001.

after the last dose was lower than the 65.6% VE against all clinical malaria during 6 months reported in the phase 3 RTS,S/AS01 study in 5–17-month-old children in the same area (Siaya County)³.

These VE data in infants contrast with >60% VE for at least 32 weeks against CHMI in US adults^{7,9} and 52% VE by time-to-event (1 – hazard ratio) and 29% VE by proportional analysis (1 – risk

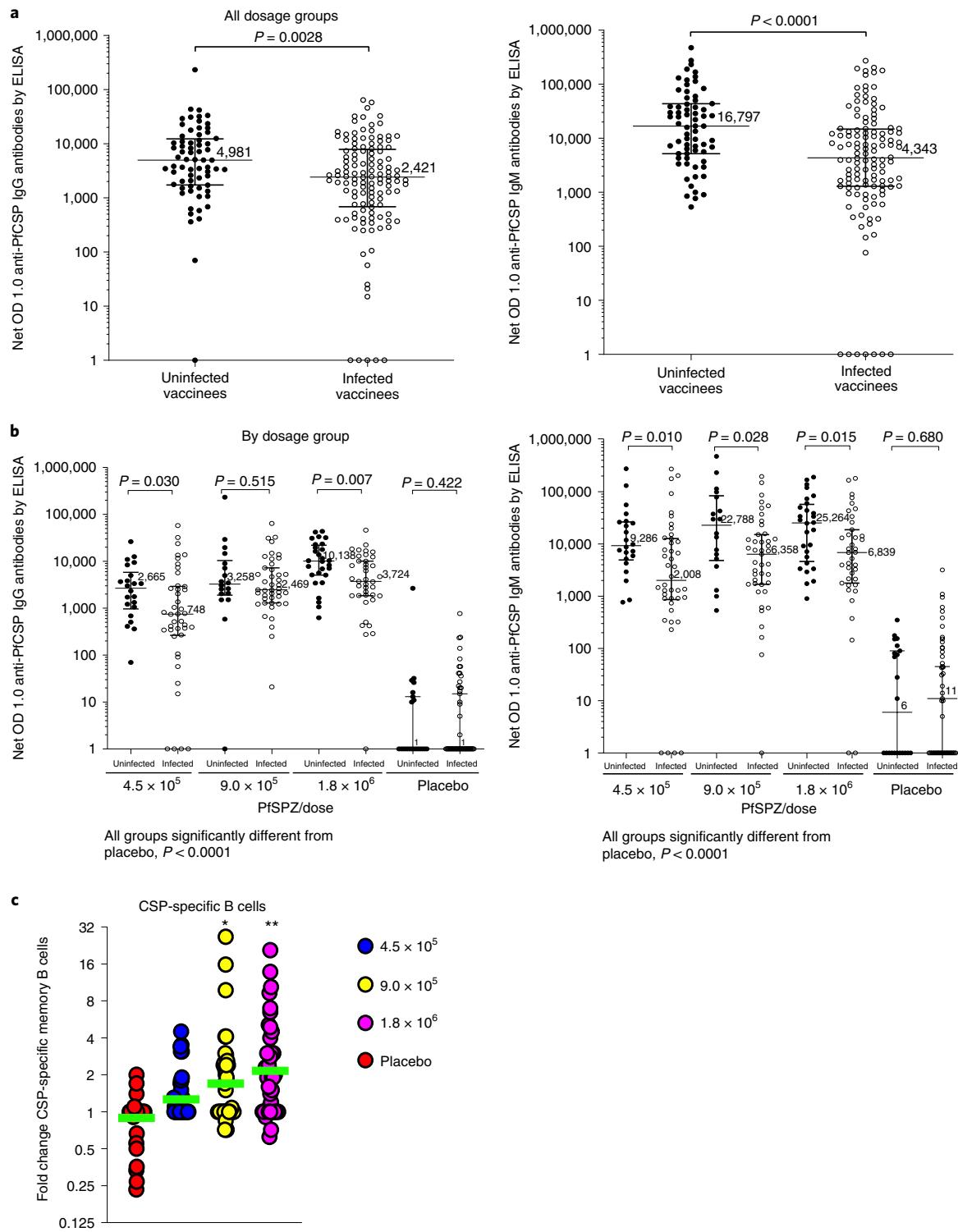


Fig. 4 | PfSPZ Vaccine-induced humoral and B cell response. **a**, Median and interquartile range of net optical density (OD) 1.0 for IgG antibodies to PfCSP 2 weeks after the last dose of PfSPZ Vaccine in infants who were uninfected (protected) and infected during the 6-month follow-up. **b**, Median and interquartile range of net optical density (OD) 1.0 for IgM antibodies to PfCSP 2 weeks after the last dose of PfSPZ Vaccine in infants who were uninfected (protected) and infected during the 6-month follow-up. **c**, B cell response to the PfSPZ Vaccine. Fold change PfCSP-specific memory B cells from preimmunization to two weeks after the third immunization of the indicated dose of PfSPZ Vaccine. The bars indicate the median values within each group. Differences among vaccine groups were assessed by one-way ANOVA with Tukey's correction for multiple comparisons. * $P < 0.05$, ** $P < 0.01$.

ratio) against malaria infection (any parasitemia detected by blood smear) at 6 months in adults in Mali¹⁷. The low-to-undetectable T cell responses in infants likely account for the lack of protection

at six months. These data are consistent with our previous study in a small number of individuals who received varying doses of the PfSPZ Vaccine showing there is a major difference in priming

PfSPZ-specific T cell responses between infants and older children and adults¹⁹. In this study, with a much larger cohort of infants, we substantiate these findings of undetectable T cell responses and provide new data indicating that this may be associated with a relative lack of Vδ2 γδ T cells at the time of the first immunization. γδ T cells respond to phosphoantigens contained in sporozoites^{27,28}, leading to their expansion and activation; thus, they provide an ‘adjuvant’ effect for T cell priming²⁹. The finding that γδ T cells are rapidly activated by sporozoites and are required to mediate the priming of T cells in mice after irradiated SPZ vaccination¹⁶ suggest this as a major possibility for the lack of T cell priming in infants. This hypothesis is further supported by adult human studies conducted in the US, which showed that Vδ2 Vγ9 cells correlate with CHMI (pre-blood stage) protection after PfSPZ vaccination^{6,7}. Although Vδ2 T cells can be generated in response to a number of different pathogens, the finding that Vδ2 T cells represent only a minor subset of the γδ T cell repertoire in infants is notable since the frequency and function of such cells correlate with protection against malaria in children living in endemic regions²³.

The importance of antibodies to PfCSP in mediating protection with the PfSPZ Vaccine has been considered secondary to T cell immunity. However, we found a dose-related increase in PfCSP-specific IgG and IgM and PfCSP-specific memory B cell response from the pre-vaccination time point to two weeks after the third vaccination (Fig. 4). We did not assess the kinetics of anti-PfCSP IgM in this study but have previously shown that they increase after each dose. Consistent with previous studies, the levels of antibodies to PfCSP two weeks after the third dose were overall significantly higher in vaccinees who were protected (did not develop infection) during the next three and six months (Fig. 4a and Extended Data Fig. 8a). This difference was consistent for each dosage group. Interestingly, at the 3- and 6-month follow-ups, the protected (uninfected) individuals in the low-dose group had lower than or similar median levels of IgG antibodies to PfCSP as the unprotected (infected) individuals in the highest-dose group (1,765 versus 3,956 (3-month) and 2,665 versus 3,724 (6-month)) (Fig. 4b and Extended Data Fig. 8b). We interpret these findings to indicate that the higher IgG antibodies within a vaccine group may be a biomarker for protection and serve as a nonmechanistic correlate.

Another potential contributing factor to the poor VE might be lack of parasite clearance before vaccinations 1 and 2 since malaria parasitemia is known to be immunosuppressive^{30–33}. In this trial, 9.6% and 15.6% of infants were parasitic by blood smear and a single quantitative PCR, respectively at the time of administration of the first dose of vaccine (Supplementary Table 3). Significant protection in African adults immunized with the PfSPZ Vaccine has only been reported in those treated with an antimalarial drug before the first dose of vaccine¹⁷. Thus, low-level *P. falciparum* parasitemia may have contributed in part to the poor VE.

This article also highlights a fundamental difference in how age may influence the T cell response to the PfSPZ Vaccine, where adults generate responses that are associated with protection while infants do not. We present hypothesis-generating data suggesting that there is an association between the frequency of Vδ2 T cells that increases with age and the generation of PfSPZ-specific T cells. We propose that future studies with the PfSPZ Vaccine should be focused on how to harness the activation of γδ T cells to enhance the priming of SPZ-specific T cell responses and protection. Overall, the data reported in this study do not currently support the application of the PfSPZ Vaccine in infant populations.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of

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Methods

Study site. This study took place in Siaya County, western Kenya from January 2017 to August 2018, a malaria-endemic area with year-round transmission and peaks during the long (April–July) and short (October–November) rainy seasons. Malaria prevalence among children aged 6 to 59 months in the study area by rapid diagnostic testing in 2017 was 62.2% (95% CI = 58.3–66.1) and 86.9% (95% CI = 84.2–89.6) of these children reported sleeping under an insecticide-treated bed net the night before the survey (A.S., personal communication). The site also took part in the RTS,S/AS01 malaria vaccine phase 3 trial in infants and children; in 2009–2010, young children aged 5–17 months in the control group of the RTS,S phase 3 trial in this area had on average more than 5 episodes of malaria per person year at risk. Before this part 2 of the trial, a dose escalation/age de-escalation part 1 of the trial was performed to get initial safety data on the 1.8×10^6 dose group in children and infants³⁵.

Participants and randomization. We recruited healthy infants who were 5–12 months old at the time of first vaccination from a 10-km radius around Siaya County Referral Hospital and Wagai Health Centre into a phase 2 multi-arm, double-blind, randomized, placebo-controlled trial. The first participant was enrolled on 23 January 2017; the last enrollment took place on 13 April 2017. Screening of participants included medical history, a comprehensive physical exam and laboratory testing for baseline hematology, renal and liver function parameters. In addition, a baseline ECG was evaluated by a pediatric cardiologist. Healthy HIV-negative infants aged 5–12 months inclusive whose parents—who were older than 18 years—consented to participation and infants who were declared able to participate for the duration of the study could be included. Exclusion criteria were a positive HIV test or breastfeeding infants of a known HIV-positive mother (per Kenyan guidelines, these HIV-exposed breastfeeding children should be on co-trimoxazole prophylaxis, which reduces malaria incidence in children by 63%³⁴) or a mother with unknown HIV status (for example, not documented in the Maternal and Child Handbook) who refused an HIV test, as well as refusal of HIV testing in the child. Children with predefined laboratory tests (elevated alanine aminotransferase $>84 \text{ U l}^{-1}$, hemoglobin $<8 \text{ g dl}^{-1}$, white blood cells $<1,500 \text{ mm}^{-3}$, neutrophils $<750 \text{ mm}^{-3}$, platelet count $<75,000 \text{ mm}^{-3}$, creatinine $>0.9 \text{ mg dl}^{-1}$) or ECG anomalies were excluded (a full list is provided in Steinhardt et al.³⁵), as were those on immunosuppressants or with splenectomy, known inherited red blood cell disorders like sickle cell disease, which is common in the area, or evidence of serious underlying conditions. A history of neurological disorder (including seizures, other than uncomplicated febrile seizures) and a known allergy to any component of the vaccine formulation, history of anaphylactic response to mosquito bites or known allergy to first- or second-line antimalarials used to treat malaria were additional exclusion criteria.

Before the start of the study, a study statistician in the US electronically generated a randomization list using permuted block randomization in the R blockrand package version 1.3. The length of each block was not disclosed to ensure blinding was fully maintained. After this schedule, a confidential treatment key linking treatment numbers to treatment assignments was prepared by the study statistician and provided to the unblinded pharmacist to be used for participant randomization at the study site. The study statistician also provided a manual randomization list that linked treatment numbers to sequence numbers, where the sequence number indicated the order of enrollment into the study. Eligible participants were randomized into one of four study groups by retrieving the next available treatment number in the manual randomization list. Once this treatment number was provided to the unblinded pharmacist, he referred to the confidential treatment key to determine the treatment assignment for that participant, consisting of PfSPZ Vaccine dosages of 4.5×10^5 , 9.0×10^5 , 1.8×10^6 of PfSPZ Vaccine or normal saline placebo at a 1:1:1 ratio.

Participants and their parents and all study staff apart from the unblinded pharmacy team, who were not involved in assessing outcomes or patient care, remained blinded for the whole period of the study. PfSPZ Vaccine and normal saline placebo cannot be distinguished by visual inspection, odor or ease of injection. Three vaccinations (or placebo injections) were administered intravenously at 8-week intervals. Before the third vaccination, all infants were treated with a full course of an artemisinin-based combination therapy (ACT), primarily artemether-lumefantrine, 11–19 d before vaccination, or, if not tolerated, dihydroartemisinin-piperaquine 19–30 d before vaccination.

Artemether-lumefantrine comes in coformulated tablets with artemether (20 mg) + lumefantrine (120 mg) per tablet. Dosing is as follows: $5 \leq 15 \text{ kg}$: 1 tablet per dose; $15 \leq 25 \text{ kg}$: 2 tablets per dose. The first and second dose were given 8 h apart; thereafter doses were given twice daily for 2 d.

Dihydroartemisinin-piperaquine is given as a coformulated pediatric tablet of 160 mg dihydroartemisinin/20 mg piperaquine, 1 tablet once daily for 3 d.

The morning dose of artemether-lumefantrine and the once-daily dose of dihydroartemisinin-piperaquine were supposed to be given under direct observation by a community interviewer; however, not all mothers complied with the instructions and reported that the medication had been given before arrival of the study staff. The study was conducted following an initial dose escalation, age de-escalation phase to establish safety of these vaccine doses in children and infants³⁵.

Safety follow-up. All safety evaluations were done in the intention-to-treat cohort, that is, in every child who received at least one PfSPZ vaccination. Infants were monitored for adverse reactions at the study clinic for 1 h after each vaccination. Solicited local (pain, pruritus, swelling, redness, bruising, induration at the injection site) and systemic (fever, inability to eat, drowsiness, irritability and allergic reaction) AEs as well as unsolicited AEs were recorded during this time and during home visits on days 2–6 postvaccination and at a clinic visit on day 8. All local AEs were considered to be related. On day 8 after vaccinations 1 and 2, and on day 14 after vaccination 3, blood samples were taken for follow-up laboratory tests (complete blood count, creatinine, alanine aminotransferase). Thereafter passive surveillance for unsolicited AEs continued until day 29 after each vaccination. SAEs were reported from the time of vaccination 1 until the end of the study. Unsolicited AEs were graded according to U.S. National Institutes of Health (NIH) Division of AIDS standards³⁴ and solicited AEs according to pediatric toxicity tables of the U.S. NIH Division of Microbiology and Infectious Diseases³⁶.

All SAEs and AEs were assessed using the following definition of levels of relatedness: definitely related: the timing and nature of the AE suggested that the vaccination/intervention was the only reason for the AE/SAE. It could not be explained by any disease or another medication; probably/likely related: AE/SAE with reasonable time relationship to vaccination or study intervention—unlikely to be attributed to disease or another drug; possibly related: AE/SAE with reasonable time relationship to vaccination or study intervention but the event could also be explained by a disease or another drug; unlikely related: AE/SAE with a time relationship to vaccination or study intervention that makes a relationship improbable but not impossible. A disease or another drug provides plausible explanation; not related: the nature and timing of the AE/SAE were not suggestive of a relationship to the vaccination/intervention and an alternative cause could be specified. Definitely, probably and possibly related were combined as ‘related’ and unlikely related and unrelated were combined as ‘unrelated’.

Malaria surveillance for efficacy evaluation. Active malaria surveillance started 2 weeks after the third vaccination through scheduled, alternating biweekly home and clinic visits and lasted 12 months postvaccination 3. During the monthly clinic visit, a blood sample was taken for detection of malaria parasites by microscopy and a dried blood spot (DBS) on filter paper was collected for molecular testing using PCR; febrile children (axillary temperature $\geq 37.5^\circ\text{C}$) and those with reported fever in the last 24 h were treated for malaria according to rapid diagnostic testing or expedited microscopy results. Scheduled interim home visits occurred two weeks after each monthly visit; if participants had fever/history of fever, they were sent to the clinic for diagnosis and treatment and a blood sample was taken. Passive surveillance included instructions to parents to bring their children to the study clinic for any illness at any time; if the child had fever/history of fever, blood samples for malaria diagnostic tests as above were taken. Positive blood smears from both active and passive surveillance were included in the analysis of the primary end point.

Laboratory analyses. *Microscopy.* Blood slides were prepared at all vaccination visits, monthly scheduled visits starting two weeks after vaccination 3 and at unscheduled visits where malaria testing was indicated due to fever or history of fever in the last 24 h. Blood smears were not read in real time unless children had fever/history of fever, in which case the smear was read immediately and children were treated if positive. Malaria infection and parasite densities were assessed using a high-power field (HPF) method with minor modifications³⁷. Thick blood film was prepared using 10 μl of blood and evenly spread in a rectangular template measuring $1 \times 2 \text{ cm}$ ($10 \times 20 \text{ mm}$). The readers traverse (PASS) through 1 cm of the film for the identification and quantitation of malaria parasites. The blood film was declared negative after scanning 1,000 HPFs (21 or 24 PASS depending on the field number of the microscope). The blood films were independently examined by two readers certified through an external quality assurance (EQA) program involving three assessments per year. The National Institute for Communicable Diseases, South Africa provided the EQA as per recommendation by the World Health Organization^{38,39}. In the case of discordant results (positive/negative discordancy; greater than twofold discrepancy if parasitemia $>400 \text{ parasites} \mu\text{l}^{-1}$; greater than tenfold discrepancy if parasitemia $<400 \text{ parasites} \mu\text{l}^{-1}$), a third read was carried out.

PCR. qPCR with reverse transcription (RT-qPCR) was performed at the same time points as blood slides using published molecular-based techniques⁴⁰ with *P. falciparum*-specific primers and probes targeting *P. falciparum* 18S ribosomal RNA gene. RT-qPCR was performed on peripheral blood samples with the inclusion in all reactions of a positive standard and a negative control with no template DNA⁴¹. All RT-qPCR assays were run from DBS samples according to standard procedures.

Immunogenicity analysis. Immunogenicity studies were performed on blood samples collected at screening and at two weeks after vaccination 3. Plasma or serum was used to measure antibodies to PfSPZ and other malaria-specific antigens by ELISA. PBMCs collected from 2.4 to 3.2 ml of blood were cryopreserved and used to assess the phenotype and function of cellular immune responses by multiparameter flow cytometry.

T and B cell staining assays. Intracellular cytokine stimulation assays were used to evaluate T cell responses elicited by the PfSPZ Vaccine as described previously⁷. Infant blood samples were collected in Kenya following the same protocol used for isolating and freezing PBMCs as the control adult samples, which were collected in the US at the NIH.⁷

Cryopreserved PBMCs were thawed using ‘thawsome’ tube adaptors⁴² and rested for 8 h. We observed no notable difference in the viability between PBMCs from Kenya and the US adult control samples. After the rest period, cells were stimulated for 17 h with 1.5×10^5 viable, irradiated, aseptic, cryopreserved PfSPZ from a single production lot or for 5 h with PMA/ionomycin (1X Cell Activation Cocktail; BioLegend) where indicated. Brefeldin A was added for the last 5 h of stimulation. B cell staining was performed on freshly thawed samples with no rest.

After stimulation, cells were stained and analyzed as described previously⁴³. Briefly, cells were washed and stained with viability dye for 20 min at room temperature, followed by surface stain for 20 min at room temperature, cell fixation and permeabilization with the BD Cytofix/Cytoperm Kit (catalog no. 554714; BD Biosciences) for 20 min at room temperature and then intracellular stain for 20 min at room temperature (see Supplementary Tables 10, 11 and 14 for a complete list of antibodies used). On completion of staining, cells were collected on a BD FACSymphony Flow Cytometer. Samples were analyzed using FlowJo v.10.6.1 (FlowJo LLC). Anomalous ‘bad’ events were separated from ‘good’ events using flowAI version 2.1⁴⁴. ‘Good events’ were used for all downstream gating. Cytokine-positive cells were determined by gating on non-naïve T cells as indicated in Extended Data Fig. 4. All antigen-specific cytokine frequencies are reported after background subtraction of identical gates from the same sample incubated with negative control stimulation (human serum albumin). Antigen-specific memory B cells were gated as indicated in Extended Data Fig. 9.

This methodology was also used for $\gamma\delta$ T cell data from an age de-escalation PfSPZ Vaccine trial in Bagamoyo, Tanzania (NCT02613520)¹⁰, which had not been previously published and is published in this article to show the age correlation of pre-vaccination $\gamma\delta$ T cell ratios (Fig. 3e).

Antibody assays. Antibody assays were conducted on sera obtained before immunization and two weeks after the third immunization. IgG antibodies to the PfCSP were assessed by ELISA as described previously¹⁰. In addition, IgM antibodies to the PfCSP were also assessed by ELISA as described; 96-well plates (Nunc Maxisorp Immuno Plates) were coated overnight at 4 °C with 2.0 µg of recombinant PfCSP protein in 50 µl per well in coating buffer (KPL). Plates were washed three times with 2 mM of imidazole, 160 mM of NaCl, 0.02% Tween 20, 0.5 mM of EDTA and blocked with 1% BSA blocking buffer (KPL) containing 1% nonfat dry milk for 1 h at 37 °C. Plates were washed three times and serially diluted serum samples (in triplicate) were added and incubated at 37 °C for 1 h. After three washes, peroxidase-labeled goat anti-human IgM (KPL) was added at a dilution of 2.0 µg ml⁻¹ and incubated at 37 °C for 1 h. Plates were washed three times, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) peroxidase substrate was added for plate development and plates were incubated for 75 min at 22 °C. Plates were read with a SpectraMax Plus 384 Microplate Reader (Molecular Devices) at 405 nm. Data were collected using SoftMax Pro GxP v.5 and fitted to a four-parameter logistic curve to calculate the serum dilution at an OD of 1.0. A negative control (pooled serum from nonimmune individuals from a malaria-free area) was included in all assays. Serum from an individual with anti-PfCSP antibodies for PfCSP was used as a positive control. For the humoral immunogenicity markers, definitions for a positive response were taken relative to the pre-dose 1 measurement. For ELISA, samples were considered positive if the difference between the postimmunization OD of 1.0 and the preimmunization OD of 1.0 (net OD = 1.0) was ≥ 50 and the ratio of the postimmunization OD of 1.0 to preimmunization OD of 1.0 (ratio) was ≥ 3.0 . The nonparametric Mann–Whitney U-test was used to determine statistical significance for fold change values of antibody levels.

Clinical and epidemiological data management. The primary efficacy end point was the incidence of *P. falciparum* parasitemia (parasite density >0) by thick blood smear in the 9.0×10^5 dose group 6 months after vaccination 3. Secondary and exploratory end points included incidence of *P. falciparum* parasitemia in each dose group compared to placebo at 3, 6, 9 and 12 months and incidence of clinical malaria (various definitions) at these time points; all trial end points are described in Supplementary Table 1. Primary analyses were done using an according-to-protocol cohort who received all three complete doses within the predefined dosing interval of 49–80 d, did not receive any other vaccine within 7 d of study vaccine/placebo, had a negative blood smear on the day of vaccination 3, did not miss more than 2 consecutive monthly clinic visits and had received ACT clearance before vaccination 3 within the specified window. All infants receiving any study product were included in an intention-to-treat cohort and were evaluated for safety and efficacy during the period of their study participation. For the according-to-protocol cohort, VE was calculated beginning two weeks after completion of the primary vaccination series; in the intention-to-treat cohort, it was calculated beginning at the time of the last vaccination.

Statistical methods. Safety, VE and immunogenicity analyses were preplanned and included in the statistical analysis plan; other analyses were exploratory. To estimate VE for the primary end point of any *P. falciparum* parasitemia, Poisson regression using robust variance estimation was used to estimate the risk ratio and VE was calculated as $1 - \text{risk ratio}$ ⁴⁵. As secondary efficacy analyses, Cox proportional-hazards models were used to assess time to first infection/clinical malaria and to analyze all clinical malaria episodes in a counting process framework⁴⁶. In the analysis of multiple episodes, the 14 d after each antimalarial treatment were subtracted from each participant’s time at risk to avoid double-counting episodes. VE was defined as $1 - \text{hazard ratio}$. Because the primary analysis was designated a priori to be VE by proportional analysis at 6 months for the 9.0×10^5 dose group, adjustment for multiple testing was not performed. Analyses were performed in R v.4.0.3; a 5% level of significance was used.

Sample size. The study was powered to detect a VE of 60% with 80% power, comparing the 9.0×10^5 dose group to placebo, assuming 30% malaria incidence in the control arm and 25% loss to follow-up, for a target sample size of 104 participants per group.

Ethical considerations. Written informed consent was obtained from the parent/guardian of each infant. The study protocol was approved by the institutional review boards of the Kenya Medical Research Institute, the Scientific and Ethics Review Unit, the US-based Centers for Disease Control and Prevention and the Kenya Pharmacy and Poisons Board and was registered at ClinicalTrials.gov (NCT02687373). An independent Data and Safety Monitoring Board (DSMB) and a local safety monitor were appointed by the trial sponsor (Sanaria). The PfSPZ Vaccine is being assessed under a U.S. Food and Drug Administration investigational new drug application and the protocol was submitted to the U.S. Food and Drug Administration more than 30 d before initiation of the clinical trial.

DSMB oversight. The independent data management team provided AE reports during the vaccination period on a weekly basis to the principal investigators and the sponsors, who would alert the DSMB in case of a safety signal as described: any SAE or any death during the 28-d vaccination period that was possibly, probably or definitely related to the study vaccination according to the principal investigators would have led to information of the DSMB, emergency unblinding for the affected participant and a decision whether vaccinations should continue, should be paused until more information became available or vaccinations should be stopped. If the same grade 3 AE, possibly to definitely related, happened in 25% or more of all participants who had been vaccinated, the safety signal would be reported by the sponsor to the DSMB, who would review all cumulative data up to this point in time. After unblinding limited to the affected participants, the DSMB would decide whether vaccinations could continue, should be paused until further information about the resolution of the AEs became available (example: raised liver function tests) or whether vaccinations would have to be discontinued permanently. If only one vaccine arm was affected by these AEs, a protocol modification may be suggested to remove the affected study dose only.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The data that support the findings of this study are available upon request to the corresponding authors and approval from Sanaria. Restrictions apply to the availability of these data, which were used under an investigational new drug application, so they are not publicly available. All requests for raw and analyzed data will be promptly reviewed by the trial sponsor, Sanaria, to verify if the request is subject to any confidentiality obligations. Patient-related data not included in the paper were generated as part of clinical trials and may be subject to patient confidentiality. Any data that can be shared will be released via a data use agreement.

Code availability

No custom code was generated for the analyses presented in this paper. Standard analysis packages and commands in the specified software were used.

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and for providing permission to publish the results. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the U.S. Centers for Disease Control and Prevention. This work was supported by the NIH Vaccine Research Center. Manufacturing and quality control release and stability assays for the PfSPZ Vaccine were supported in part by the National Institute of Allergy and Infectious Diseases, NIH (Small Business Innovation Research grant nos. 5R44AI055229-09A1 and 2R44AI058375-06A1 to S.L.H.).

Author contributions

M.O., L.C.S., R.Y., S.L.H., T.L.R. and R.A.S. planned the study. M.O., R.Y., D.A., T.S., E.L.N., A.D., P.N.O., K.O., D.K.B., S.K., W.C. and A.M.S. conducted the study. B.K.L.S., E.R.J., N.K.C., P.F.B., T.L.R. and S.L.H. provided the study product. P.A.S.II, R.A.S., K.O., W.C. and N.K.C. conducted the laboratory and immunogenicity analyses. G.A. analyzed all the ECGs. R.E.W., D.S. and G.E.P. analyzed the data. S.J., S.A., C.D. and M.M. provided samples for the immunogenicity data. M.O., L.C.S., K.O., R.E.W., J.R.G., T.S., S.K., A.M.S., T.L.R., B.K.L.S., S.L.H., P.A.S.II and R.A.S. interpreted the study results. M.O., L.C.S., S.L.H., T.L.R., P.A.S.II and R.A.S. wrote the paper. All authors reviewed, edited and approved the paper.

Competing interests

T.L.R., N.K.C., P.F.B., E.R.J., B.K.L.S. and S.L.H. are employees of Sanaria, which manufactures the vaccine tested in this study. S.L.H. and B.K.L.S. are named inventors on patents related to the PfSPZ Vaccine. All other authors report no potential conflicts of interest.

Additional information

Extended data is available for this paper at <https://doi.org/10.1038/s41591-021-01470-y>.

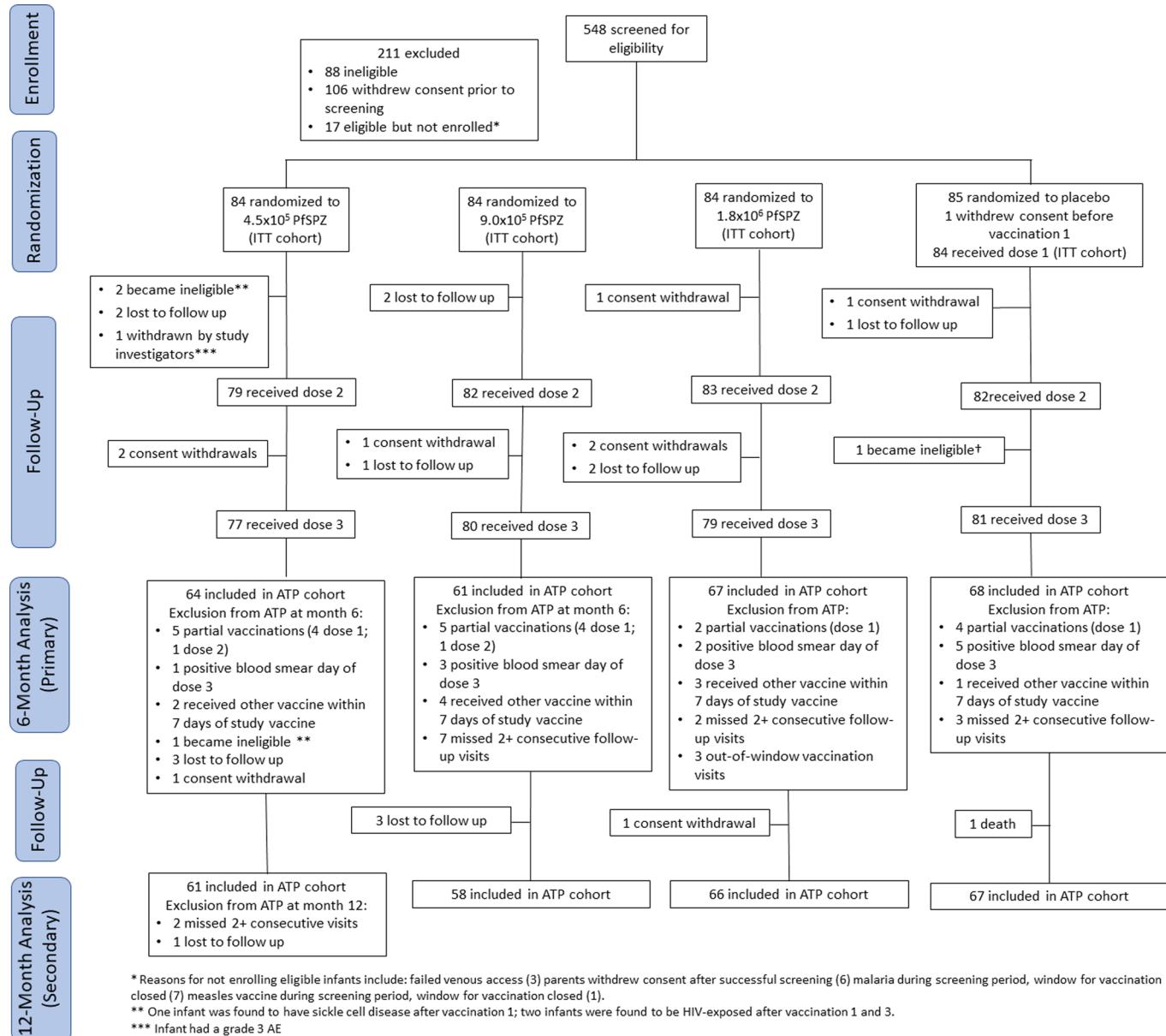
Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41591-021-01470-y>.

Correspondence and requests for materials should be addressed to Martina Onoko or Robert A. Seder.

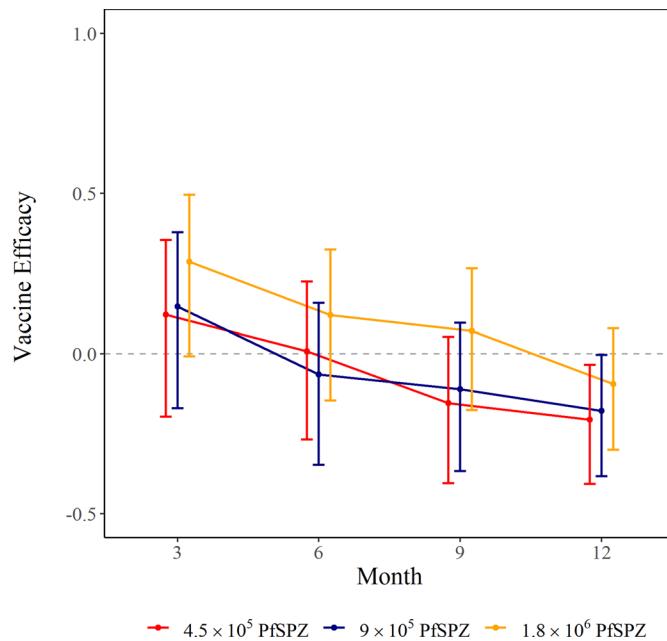
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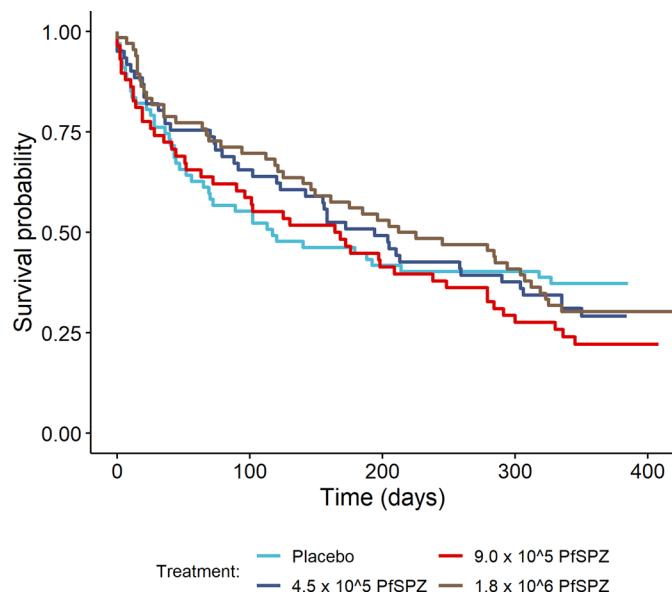
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Extended Data Fig. 1 | Trial Profile. Trial enrolment and participation details.

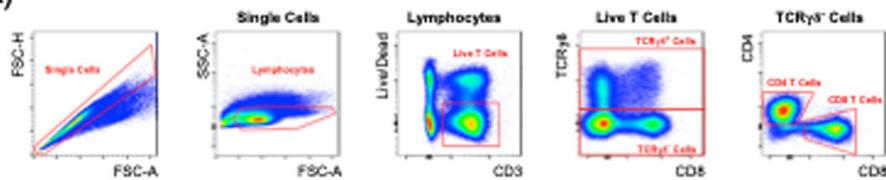


Extended Data Fig. 2 | Proportional efficacy of PfSPZ Vaccine over time. Proportional efficacy of various doses of PfSPZ Vaccine against Plasmodium falciparum parasitemia (>0 parasites/ μ L) at 3, 6, 9, and 12 months follow-up.

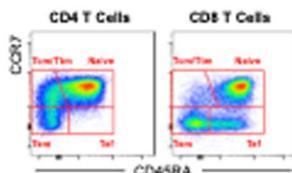


Extended Data Fig. 3 | Survival analysis of first/only clinical malaria. Kaplan-Meier survival analysis of time-to first or only clinical malaria episode over 12 months, by treatment group.

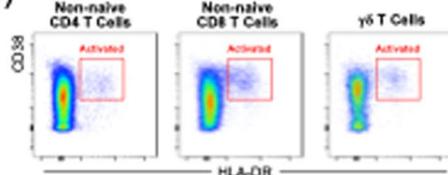
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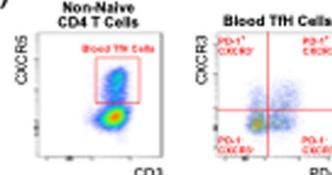
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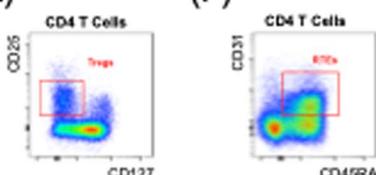
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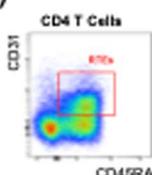
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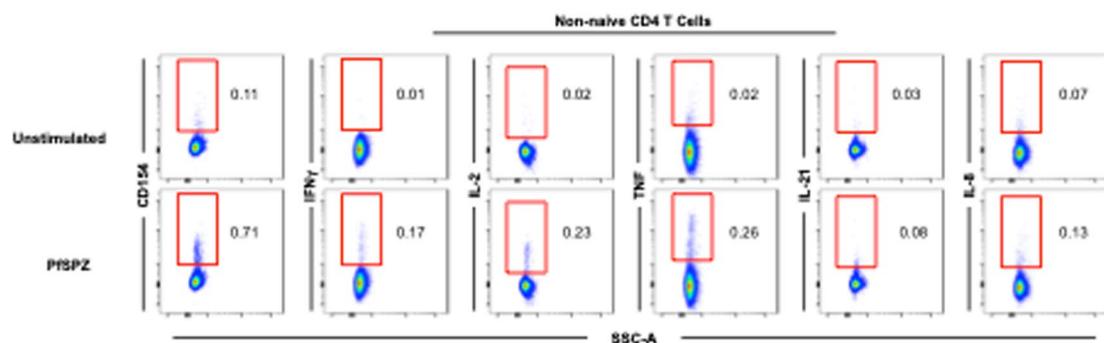
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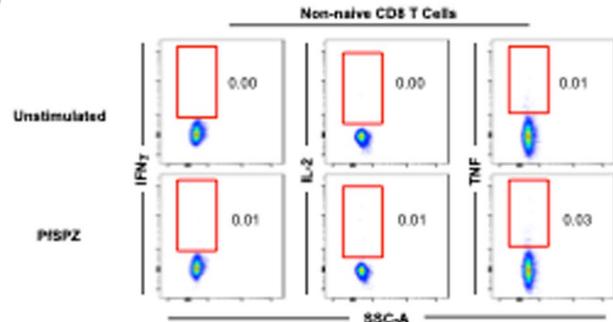
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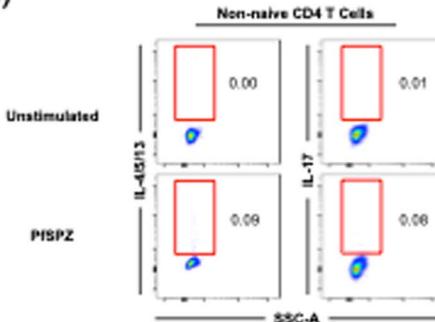
(G)



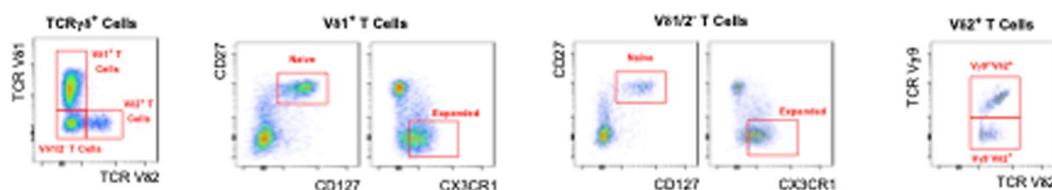
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(I)

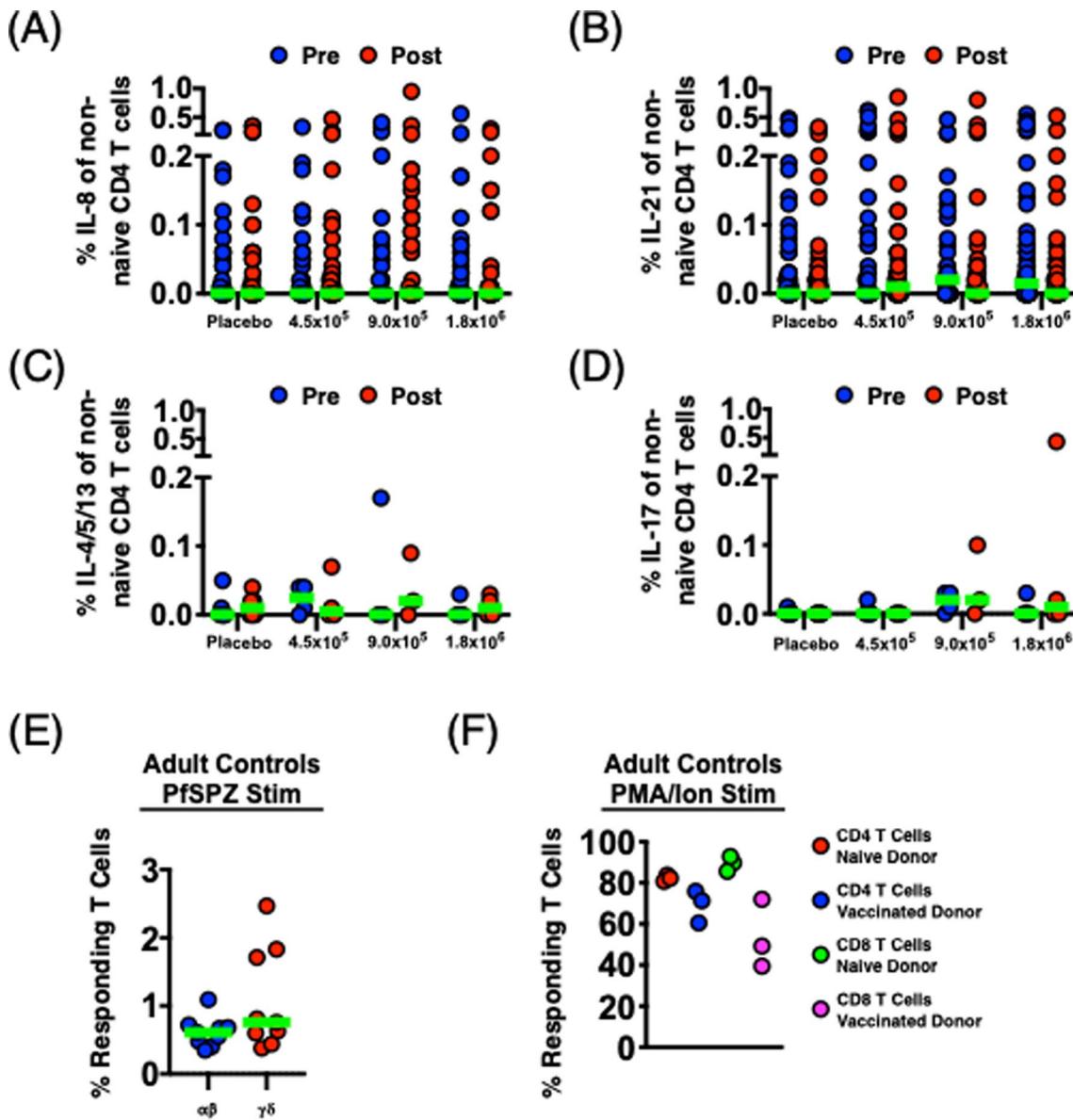


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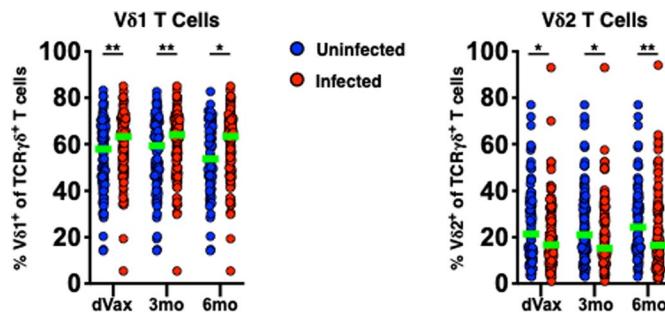


Extended Data Fig. 4 | See next page for caption.

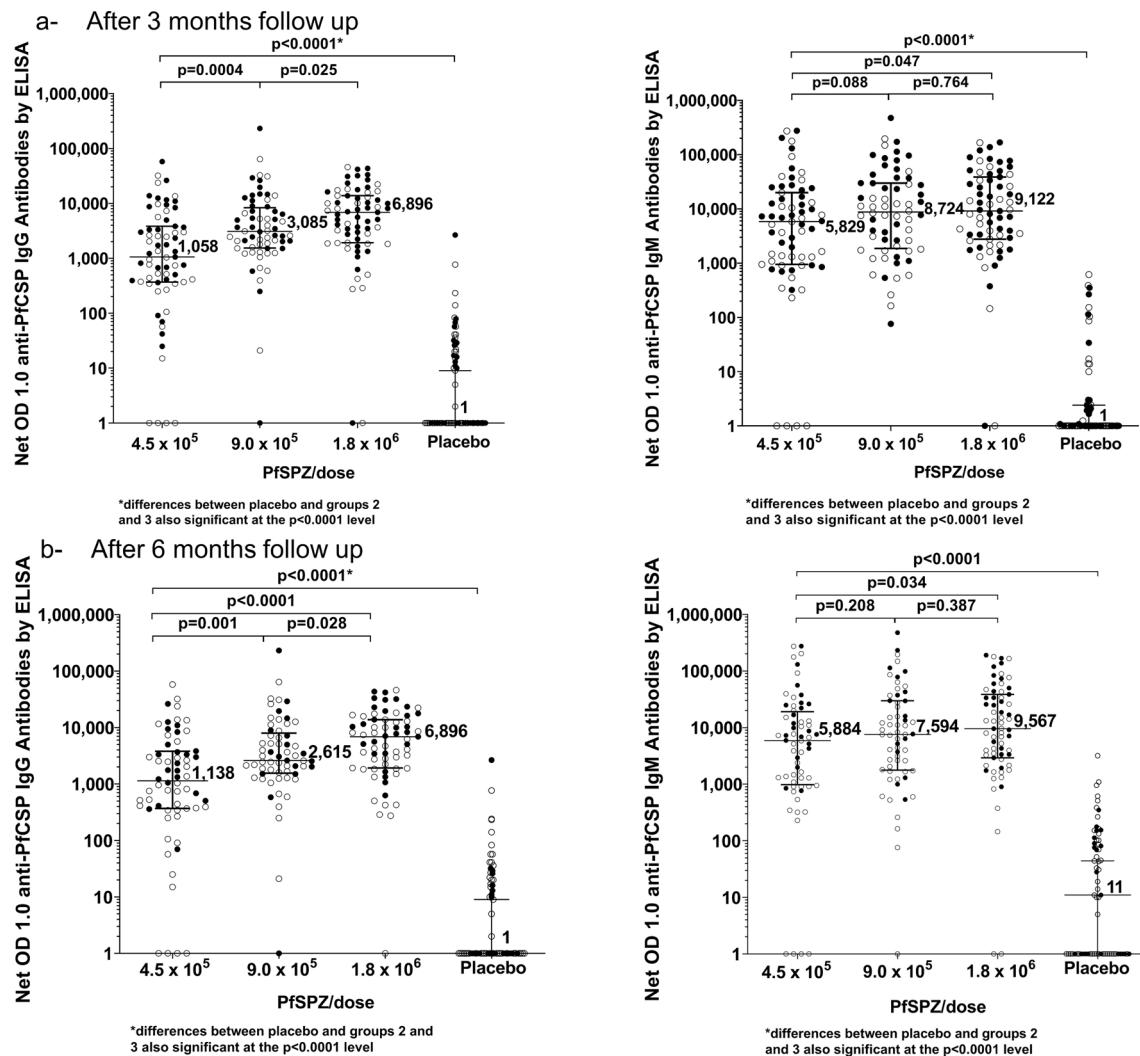
Extended Data Fig. 4 | Flow cytometry gating for analysis of T cells. (a–g) Cellular gating tree using the 28-color panel outlined in Supplementary Information Tables 10 and 11. (a) Lineage gating of T cells. After gating on single cells, lymphocytes, and live CD3⁺ T cells, a pan-TCR $\gamma\delta$ antibody was used to separate $\gamma\delta$ and $\alpha\beta$ T cells, which were further subdivided into CD4⁺ and CD8⁺ $\alpha\beta$ T cells. (b) CCR7 and CD45RA antibodies were used to gate CD4⁺ and CD8⁺ $\alpha\beta$ T cell naïve and memory subsets. Naive T cells (naïve), Central and transitional memory T cells (Tcm/Ttm), Effector memory T cells (Tem), and Effector T cells (Tef). (c) CD38 and HLA-DR were used to identify activated non-naïve CD4 and CD8 $\alpha\beta$ T cells and $\gamma\delta$ T cells. (d) CXCR5⁺ was used to identify blood T follicular helper (Tfh) cells of non-naïve CD4 T cells, and blood Tfh subsets were further characterized using PD-1 and CXCR3. (e) CD25 and CD127 were used to identify T regulatory cells (Tregs). (f) CD31 and CD45RA were used to identify recent thymic emigrants (RTEs) of CD4 T cells. (g–i) Gating to measure T cell function following no stimulation or stimulation with PFSZ. The panel used in Supplementary Table 10 was used for the functional gating of non-naïve CD4 and CD8 T cells in (g) and (h), respectively, while the panel in Supplementary Table 11 was used for the Th2 and Th17 gating of non-naïve CD4 T cells in (i). (j) TCR V δ 1 and V δ 2 antibodies were used to identify $\gamma\delta$ T cell subsets. V δ 1 and V δ 1/V δ 2⁺ T cell subsets were further analyzed for naïve (CD27⁺CD127⁺) vs clonal expansion (CX3CR1⁺CD127⁻). V γ 9 was used to identify V γ 9⁺ or V γ 9⁻V δ 2⁺ T cells.

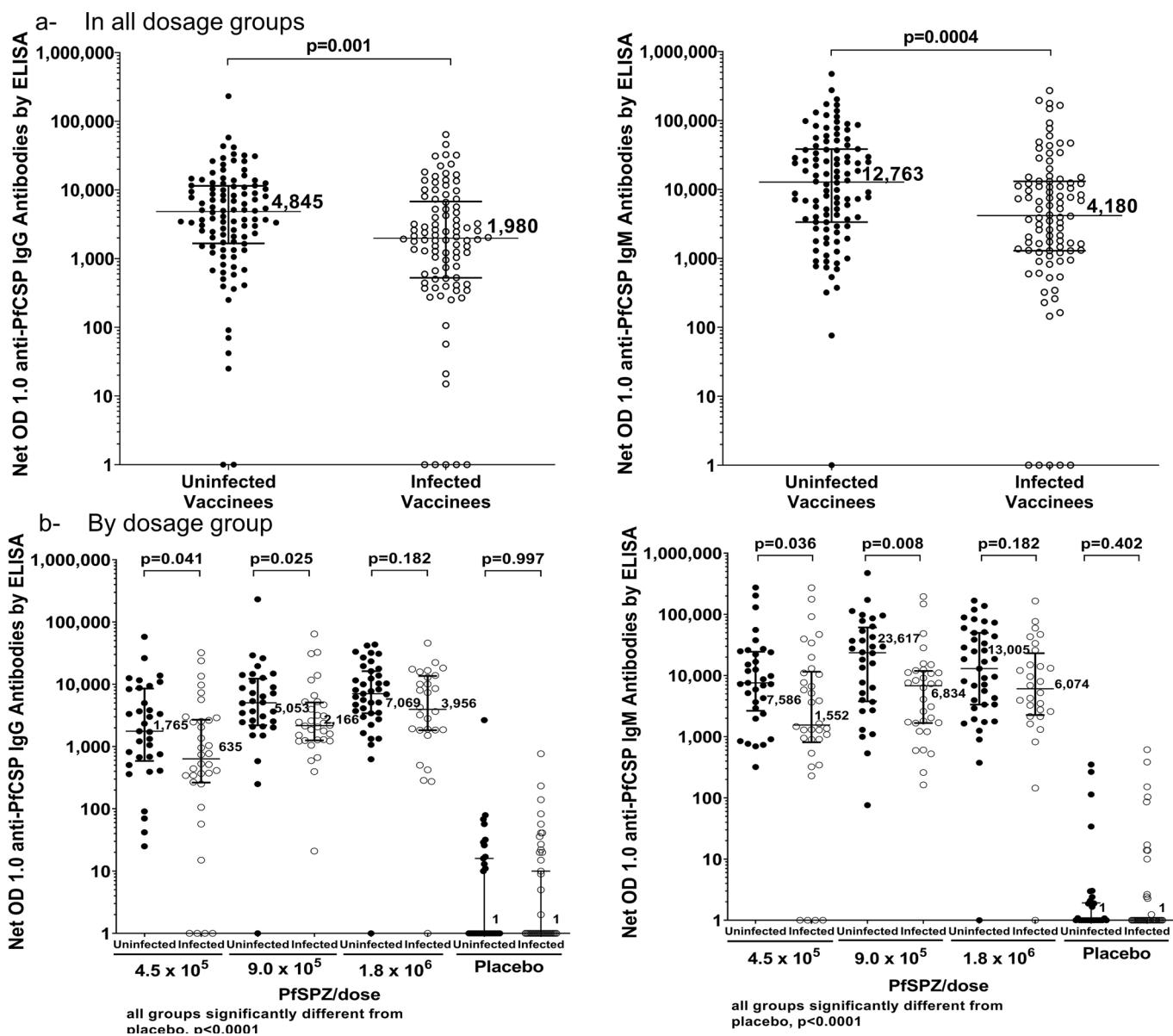


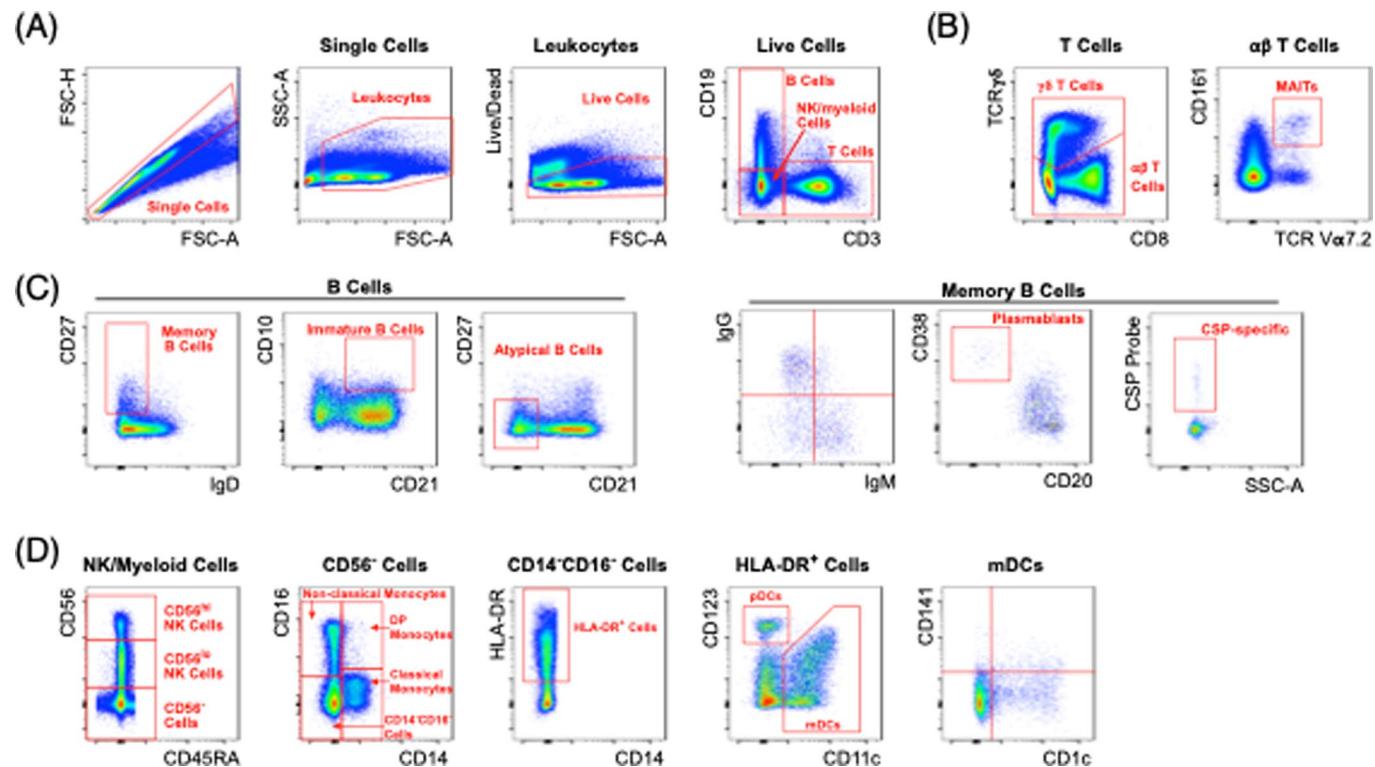
Extended Data Fig. 5 | PfSPZ Vaccine-induced T cell responses. (a-d) T cell response to PfSPZ Vaccine. Percent of non-naïve CD4 T cells in the blood expressing IL-8 (a), IL-21 (b), Th2 cytokines (c), or IL-17 (d) at pre-immunization (blue) or 2 weeks after the 3rd immunization (red) of the indicated dose of PfSPZ Vaccine. Results are the percentage of cytokine-producing cells after incubation with PfSPZ minus the percentage of cytokine-producing cells after incubation with vaccine diluent (medium with 1% human serum albumin). Bars indicate median values within each group. Bars indicate median values and differences at each timepoint between pre and post vaccination groups were assessed using multiple T tests with Holm-Sidak's correction for multiple comparisons. (e) A single PfSPZ-vaccinated adult PBMC sample was included in each batch as a positive control for PfSPZ stimulation. Each symbol represents the percent of non-naïve CD4 T cells expressing CD154, IFN γ , IL-2, or TNF (blue) or $\gamma\delta$ T cells expressing IFN γ , IL-2, or TNF (red) in the positive control sample within a single batch. Green bars indicate median values. (f) Percent of non-naïve CD4 T cells expressing CD154, IFN γ , IL-2, or TNF or CD8 T cells expressing IFN γ , IL-2, or TNF in adult control samples following PMA/ionomycin stimulation.



Extended Data Fig. 6 | Infection susceptibility correlates with pre-vaccination $\gamma\delta$ T cell frequencies. Pre-vaccination frequencies of V δ 1 (left) and V δ 2 (right) $\gamma\delta$ T cell populations in subjects that were either uninfected (blue) or infected (red) during the vaccination period (dVax), during 3-month ATP, or during 6-month ATP. Differences at each timepoint between pre and post vaccination groups were assessed using multiple T tests with Holm-Sidak's correction for multiple comparisons. *P<0.05, **P<0.01.







Extended Data Fig. 9 | Flow cytometry gating for phenotyping analysis of B/innate immune cells. Cellular gating tree using the 25-color panel outlined in Supplementary Table 4. (A) Lineage gating includes gating on single cells, leukocytes, live cells, and then CD19 and CD3 are used to separate live B cells, T cells, and NK/myeloid cells. (B) T cell gating to identify $\gamma\delta$ T cells (pan- $\gamma\delta$ TCR) and mucosal-associated invariant T (MAIT) cells (CD161+TCRV α 7.2+). (C) Gating for B cell populations. B cells were further divided into memory B cells (CD27+IgD+), immature B cells (CD10+CD21+), and atypical B cells (CD27-CD21+). Memory B cells were further divided into plasmablasts (CD38+CD20-), plasmodium (CSP)-specific B cells (CSP-probe+), and B cell isotypes using IgG and IgM antibodies. (D) Gating tree to identify various subsets of NK/myeloid cell populations. NK cell populations were first divided by CD56 expression. From the CD56- cell gate, monocyte subsets were characterized using CD14 and CD16. CD14-CD16- that were HLA-DR+ were used to identify dendritic cell (DC) populations including plasmacytoid DCs (pDCs) (CD123+CD11c-) myeloid-derived DCs (mDCs) (CD123-CD11c+). mDCs subsets were identified using CD1c and CD141.

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Data collection

After being recorded on paper forms, clinical data (including adverse events, concomitant medications, and reactogenicity data) were submitted into a 21 CFR Part 11-compliant Internet Data Entry System (IDES) called AdvantageEDC provided by The Emmes Corporation. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate.

Data analysis

Analyses were done in SAS version 9.4 (SAS Institute, Cary NC), and R version 4.0.3. [1]
[1] R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.

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Sample size

The study was powered to detect a VE of 60% with 80% power, comparing the 9.0×10^5 dose group to placebo, assuming 30% malaria incidence in the control arm and 25% loss to follow-up, for a target sample size of 104 participants/group.

Data exclusions

Primary analyses were done using an according-to-protocol (ATP) cohort who received all three complete doses within the pre-defined dosing interval of 49–80 days, did not receive any other vaccine within 7 days of study vaccine/placebo, had a negative blood smear on day of vaccination 3, did not miss more than 2 consecutive monthly clinic visits and had received the ACT clearance before vaccination 3 within the specified window. The exclusion criteria were included in the protocol, except for the amount of missed clinic visits, as investigators wanted to see the distribution of these data before determining the relevant criterion.

Replication

All methods have been carefully documented in the protocol and included in the manuscript. The trial was not replicated but could be from the documented descriptions of the methods.

Randomization

Before the start of the study, a study statistician in the US electronically generated a randomization list using permuted-block randomization in the R `blockrand` package (R foundation for statistical computing, Vienna, Austria). The length of each block was not disclosed to ensure blinding is fully maintained. Following this schedule, a confidential treatment key linking treatment numbers to treatment assignments was prepared by the study statistician and provided to the unblinded pharmacist to be used for subject randomization at the study site. The study statistician also provided a Manual Randomization List which linked the treatment numbers to sequence numbers, where the sequence number indicated the order of enrollment into the study. Eligible participants were randomized into one of four study groups by retrieving the next available treatment number in the Manual Randomization List. Once this treatment number was provided to the unblinded pharmacist, he referred to the confidential treatment key to determine the treatment assignment for that participant, consisting of Eligible participants were randomized using permuted block randomization in R (R foundation for statistical computing, Vienna, Austria) to receive PfSPZ Vaccine dosages of 4.5×10^5 , 9.0×10^5 , 1.8×10^6 PfSPZ of PfSPZ Vaccine or normal saline placebo in a 1:1:1:1 ratio.

Blinding

Participants and their parents and all study staff apart from the unblinded pharmacy team, which was not involved in assessing outcomes or patient care, remained blinded for the whole period of the study.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

| | |
|-------------------------------------|-----------------------------------------------------------------|
| n/a | Involved in the study |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Antibodies |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Eukaryotic cell lines |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology and archaeology |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Animals and other organisms |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> Human research participants |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> Clinical data |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Dual use research of concern |

Methods

| | |
|-------------------------------------|----------------------------------------------------|
| n/a | Involved in the study |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> Flow cytometry |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging |

Human research participants

Policy information about [studies involving human research participants](#)

Population characteristics

We recruited healthy infants who were 5–12 months old at the time of first vaccination from a 10-kilometer radius around Siaya County Referral Hospital and Wagai Health Centre.

Recruitment

We recruited healthy infants who were 5–12 months old at the time of first vaccination from a 10-kilometer radius around Siaya County Referral Hospital and Wagai Health Centre into a phase 2 double-blinded, randomized, placebo-controlled trial. Eighty-four children were randomized to each of the four study arms, and the mean age per group ranged from 8.0 to 8.5.

months. Each group had between 38.1% to 53.6% female participants, and at least 92.9% of each group was ethnically Luo.

Ethics oversight

The study protocol was approved by institutional review boards of the Kenya Medical Research Institute (KEMRI), the US-based Centers for Disease Control and Prevention (CDC), and the Kenya Pharmacy and Poisons Board (PPB).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration

ClinicalTrials.gov Identification number: NCT02687373

Study protocol

The full study protocol is being submitted along with the manuscript and is also available from the corresponding author upon request.

Data collection

This study took place in Siaya County, western Kenya from January 2017 to August 2018, a malaria-endemic area with year-round transmission and peaks during the long (April–July) and short (October–November) rainy seasons. Recruitment took place from January through May 2017, with follow-up of participants until August of 2018.

Outcomes

Primary and secondary outcomes were pre-defined in the study protocol. They were measured using standardized laboratory methods, including thick and thin blood smears, polymerase chain reaction, and ELISAs.

Flow Cytometry

Plots

Confirm that:

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

PBMCs ficoll-purified from whole blood.

Instrument

BD FACSymphony Cell Analyzer

Software

FlowJo version 10.6.1

Cell population abundance

N/A

Gating strategy

See figures SI Figure 4 and SI Figure 9.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.